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DT05 Rec'd PCT/PTO 09 FEB 2005

DESCRIPTION

METHOD OF DIAGNOSING RISK OF RESTENOSIS AFTER CORONARY ANGIOPLASTY

TECHNICAL FIELD

The present invention relates to a detection method using genes associated with restenosis after coronary angioplasty. More particularly, it relates to a detection method using a plurality of gene polymorphisms associated with restenosis after coronary angioplasty and to a kit used for the method. The present invention can be used for, for example, diagnosing a risk of restenosis after coronary angioplasty.

BACKGROUND ART

Although coronary angioplasties have been carried out widely as a treatment for coronary artery diseases, restenosis is a large problem (McBride W, Lange RA, Hillis LD. Restenosis after successful coronary angioplasty. Pathophysiology and prevention. N Engl J Med 1998;318:1734-7). The use of intra-coronary stents reduces the incidence of restenosis, however, restenosis is still observed in 10 to 20% (Serruys PW, de Jaegere P, Kiemeneij F, et al. A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. Benestent Study Group. N Engl J Med 1994;331:489-95.). A number of clinical and angiographic findings, including hypertension, diabetes mellitus, hyperlipidemia, unstable angina, severe coronary artery stenosis and long stenosis lesions, have been reported to be associated with an increased risk of restenosis after coronary angioplasty (Hirshfeld JW Jr, Schwartz JS, Jugo R, et al. Restenosis after coronary angioplasty: a multivariate statistical model to relate lesion and procedure variables to restenosis. The M-HEART Investigators. J Am Coll Cardiol 1991;18:647-56.; Weintraub WS, Kosinski AS, Brown CL 3rd, King SB 3rd. Can restenosis after coronary angioplasty be predicted from clinical variables? J Am Coll Cardiol 1993;21:6-14.; Stein B, Weintraub WS, Gebhart SP, et al. Influence of diabetes mellitus on early and late outcome after percutaneous transluminal coronary angioplasty. Circulation 1995;91:979-89.; Violaris AG, Melkert R, Serruys PW. Long-term luminal renarrowing after successful elective coronary angioplasty of total occlusions. A quantitative angiographic analysis.

Circulation 1995;91:2140-50.). The molecular mechanisms underlying restenosis, however, remain to be elucidated. Intra-coronary ultrasound studies in humans suggest that chronic remodeling (vascular constriction) is the major mechanism of restenosis after balloon dilatation (Mintz GS, Popma JJ, Pichard AD, et al. Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study. Circulation 1996;94:35-43.), whereas neointimal hyperplasia is the most important mechanism in in-stent restenosis (Hoffmann R, Mintz GS, Dussaillant GR, et al. Patterns and mechanisms of in-stent restenosis. A serial intravascular ultrasound study. Circulation 1996;94:1247-54). One approach to preventing the development of restenosis after coronary angioplasty is to identify susceptibility genes. Although genetic epidemiological studies have revealed that several genetic polymorphims, including those of angiotensin-converting enzyme (Amant C, Bauters C, Bodart J-C, et al. *D* allele of the angiotensin I-converting enzyme is a major risk factor for restenosis after coronary stenting. Circulation 1997;96:56-60.; Ribichini F, Steffenino G, Dellavalle A, et al. Plasma activity and insertion/deletion polymorphism of angiotensin I-converting enzyme: a major risk factor and a marker of risk for coronary stent restenosis. Circulation 1998;97:147-154.), angiotensinogen (Volzke H, Hertwig S, Rettig R, Motz W. The angiotensinogen gene 235T variant is associated with an increased risk of restenosis after percutaneous transluminal coronary angioplasty. Clin Sci 2000;99:19-25.), apolipoprotein E (van Bockxmeer FM, Mamotte CDS, Gibbons FR, Taylor RR. Apolipoprotein e4 homozygosity-a determinant of restenosis after coronary angioplasty. Atherosclerosis 1994;110:195-202.), platelet glycoprotein IIIa (Walter DH, Schachinger V, Elsner M, Dimmeler S, Zeiher AM. Platelet glycoprotein IIIa polymorphisms and risk of coronary stent thrombosis. Lancet 1997; 350: 1217-1219.), and stromelysin-1 (Humphries S, Bauters C, Meirhaeghe A, Luong L, Bertrand M, Amouyel P. The 5A6A polymorphism in the promoter of the stromelysin-1 (MMP3) as a risk factor for restenosis. Eur Heart J 2002;23:721-725.), have been reported to be associated with restenosis after balloon dilatation or in-stent restenosis, the genes that contribute to genetic susceptibility to restenosis remain to be identified definitively.

SUMMARY OF THE INVENTION

As mentioned above, a large number of association study between gene polymorphisms and restenosis after coronary angioplasty have been carried out previously. However, many studies have not reached a certain finding in terms of

significance thereof. This is mainly because populations of subjects in many studies are not sufficient and not only gene polymorphisms but also environmental factors are different between races. Furthermore, even if the association with restenosis is recognized, in the analysis of large scale population, relative risk (odds ratio) is generally low.

The present invention was made on the basis of the above- mentioned background, and the object thereof is to provide a means of diagnosing genetic risk of restenosis after coronary angioplasty with high accuracy and high predictability.

To achieve the above-mentioned objects, the present inventors have extracted 71 genes which were estimated to be associated with coronary arteriosclerosis, coronary artery spasm, hypertension, diabetes mellitus, hyperlipidemia, etc., and mainly selected 112 polymorphisms which were predicted to be associated with functional changes of genes by the use of a plurality of public databases. Then, as to 112 polymorphisms of 71 genes, association study with respect to myocardial infarction was carried out in 445 myocardial cases and 464 controls. As a result, the present inventors have found 19 SNPs (single nucleotide polymorphisms) which were associated with myocardial infarction in men and 18 SNPs in women (Yamada Y, Izawa H, Ichihara S, et al. Genetic risk diagnosis system for myocardial infarction developed by a large scale association study of 112 gene polymorphisms in 5061 individuals (in press)). However, such polymorphisms also inluded candidate genes of restenosis after coronary angioplasty. Then, the present inventors performed a large scale association study on the association between these SNPs and restenosis after cornoary angioplasty. As a result, the present inventors succeeded in identifying ten SNPs which were associated with restenosis after coronary angioplasty in men and seven SNPs in women. In addition, analysis of the combination of these polymorphisms revealed maximal odds ratios of 15.09 and 44.54 for restenosis after balloon dilatation, and of 6.64 and 117.83 for in-stent restenosis in men and women, respectively, on the basis of the stepwise forward selection method of multivariate logistic regression analysis. In the analysis, the odds ratios were maximum among the odds ratios which had been reported in the past. Based on these results, it was possible to obtain a finding that by selecting a plurality of SNPs from these SNPs and using the combination of the results of analysis of each SNP, diagnosis of restenosis after coronary angioplasty can be carried out with high reliability and high predictability. The

present invention was made based on the above findings and provides the following configuration.

[1] A method for detecting the genotype in a nucleic acid sample, comprising the following step (a):

(a) analyzing two or more polymorphisms selected from the group consisting of the following (1) to (6) in a nucleic acid sample:

(1) polymorphism at the base number position 3932 of the apolipoprotein E gene;

(2) polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(3) polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(4) polymorphism at the base number position 825 of G-protein β3 subunit gene;

(5) polymorphism at the base number position -482 of the apolipoprotein C-III gene; and

(6) polymorphism at the base number position -6 of the angiotensinogen gene.

[2] A method for detecting the genotype in a nucleic acid sample, comprising the following step (b):

(b) analyzing two or more polymorphisms selected from the group consisting of the following (7) to (11) in a nucleic acid sample:

(7) polymorphism at the base number position 1186 of the thrombospondin 4 gene;

(8) polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(9) polymorphism at the base number position 2136 of the thrombomodulin gene;

(10) polymorphism at the base number position 5713 of the thrombopoietin gene; and

(11) polymorphism at the base number position 994 of the platelet-activating factor acetylhydrolase gene.

[3] A method for detecting the genotype in a nucleic acid sample, comprising the

following step (c):

(c) analyzing two or more polymorphisms selected from the group consisting of the following (12) to (17) in a nucleic acid sample:

(12) polymorphism at the base number position 561 of the E-selectin gene;

(13) polymorphism at the base number position 2445 of the fatty acid-binding protein 2 gene;

(14) polymorphism at the base number position 1018 of the glycoprotein Ibα gene;

(15) polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(16) polymorphism at the base number position 584 of the paraoxonase gene; and

(17) polymorphism at the base number position 3932 of the apolipoprotein E gene.

[4] A method for detecting the genotype in a nucleic acid sample, comprising the following step (d):

(d) analyzing two or more polymorphisms selected from the group consisting of the following (18) to (22) in a nucleic acid sample:

(18) polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(19) polymorphism at the base number position -482 of the apolipoprotein C-III gene;

(20) polymorphism at the base number position 584 of the paraoxonase gene;

(21) polymorphism at the base number position 1018 of glycoprotein Ibα gene; and

(22) polymorphism at the base number position 3932 of the apolipoprotein E gene.

[5] A method for diagnosing the risk of restenosis after coronary angioplasty, comprising the following steps (i) to (iii):

(i) analyzing two or more polymorphisms selected from the group consisting of the following (1) to (6) in a nucleic acid sample:

(1) polymorphism at the base number position 3932 of the apolipoprotein E gene;

(2) polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(3) polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(4) polymorphism at the base number position 825 of G-protein β3 subunit gene;

(5) polymorphism at the base number position -482 of the apolipoprotein C-III gene; and

(6) polymorphism at the base number position -6 of the angiotensinogen gene;

(ii) determining, based on the information about polymorphism which was obtained in the step (i), the genotype of the nucleic acid sample; and

(iii) assessing, based on the genotype determined, a genetic risk of restenosis after coronary angioplasty.

[6] A method for diagnosing the risk of restenosis after coronary angioplasty, comprising the following steps (iv) to (vi):

(iv) analyzing two or more polymorphisms selected from the group consisting of the following (7) to (11) in a nucleic acid sample:

(7) polymorphism at the base number position 1186 of the thrombospondin 4 gene;

(8) polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(9) polymorphism at the base number position 2136 of the thrombomodulin gene;

(10) polymorphism at the base number position 5713 of the thrombopoietin gene; and

(11) polymorphism at the base number position 994 of the platelet-activating factor acetylhydrolase gene;

(v) determining, based on the information about polymorphism which was obtained in the step (iv), the genotype of the nucleic acid sample; and

(vi) assessing, based on the genotype determined, a genetic risk of restenosis after coronary angioplasty.

[7] A method for diagnosing the risk of restenosis after coronary angioplasty, comprising the following steps (vii) to (ix):

(vii) analyzing two or more polymorphisms selected from the group consisting of the following (12) to (17) in a nucleic acid sample:

(12) polymorphism at the base number position 561 of the E-selectin gene;

(13) polymorphism at the base number position 2445 of the fatty acid-binding protein 2 gene;

(14) polymorphism at the base number position 1018 of the glycoprotein Ibα gene;

(15) polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(16) polymorphism at the base number position 584 of the paraoxonase gene; and

(17) polymorphism at the base number position 3932 of the apolipoprotein E gene;

(viii) determining, based on the information about polymorphism which was obtained in the step (vii), the genotype of the nucleic acid sample; and

(ix) assessing, based on the genotype determined, a genetic risk of restenosis after coronary angioplasty.

[8] A method for diagnosing the risk of restenosis after coronary angioplasty, comprising the following steps (x) to (xii):

(x) analyzing two or more polymorphisms selected from the group consisting of the following (18) to (22) in a nucleic acid sample:

(18) polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(19) polymorphism at the base number position -482 of the apolipoprotein C-III gene;

(20) polymorphism at the base number position 584 of the paraoxonase gene;

(21) polymorphism at the base number position 1018 of glycoprotein Ibα gene; and

(22) polymorphism at the base number position 3932 of the

apolipoprotein E gene;

(xi) determining, based on the information about polymorphism which was obtained in the step (x), the genotype of the nucleic acid sample; and

(xii) assessing, based on the genotype determined, a genetic risk of restenosis after coronary angioplasty.

[9] A kit for detecting the genotype, comprising two or more of nucleic acids selected from the group consisting of the following (1) to (6):

(1) a nucleic acid for analyzing polymorphism at the base number position 3932 of the apolipoprotein E gene;

(2) a nucleic acid for analyzing polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(3) a nucleic acid for analyzing polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(4) a nucleic acid for analyzing polymorphism at the base number position 825 of G-protein β3 subunit gene;

(5) a nucleic acid for analyzing polymorphism at the base number position -482 of the apolipoprotein C-III gene; and

(6) a nucleic acid for analyzing polymorphism at the base number position -6 of the angiotensinogen gene.

[10] A kit for detecting the genotype, comprising two or more of nucleic acids selected from the group consisting of the following (7) to (11):

(7) a nucleic acid for analyzing polymorphism at the base number position 1186 of the thrombospondin 4 gene;

(8) a nucleic acid for analyzing polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(9) a nucleic acid for analyzing polymorphism at the base number position 2136 of the thrombomodulin gene;

(10) a nucleic acid for analyzing polymorphism at the base number position 5713 of the thrombopoietin gene; and

(11) a nucleic acid for analyzing polymorphism at the base number position 994 of the platelet-activating factor acetylhydrolase gene.

[11] A kit for detecting the genotype, comprising two or more of nucleic acids

selected from the group consisting of the following (12) to (17):

(12) a nucleic acid for analyzing polymorphism at the base number position 561 of the E-selectin gene;

(13) a nucleic acid for analyzing polymorphism at the base number position 2445 of the fatty acid-binding protein 2 gene;

(14) a nucleic acid for analyzing polymorphism at the base number position 1018 of glycoprotein Ibα gene;

(15) a nucleic acid for analyzing polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(16) a nucleic acid for analyzing polymorphism at the base number position 584 of the paraoxonase gene; and

(17) a nucleic acid for analyzing polymorphism at the base number position 3932 of the apolipoprotein E gene.

[12] A kit for detecting the genotype, comprising two or more of nucleic acids selected from the group consisting of the following (18) to (22):

(18) a nucleic acid for analyzing polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(19) a nucleic acid for analyzing polymorphism at the base number position -482 of the apolipoprotein C-III gene;

(20) a nucleic acid for analyzing polymorphism at the base number position 584 of the paraoxonase gene;

(21) a nucleic acid for analyzing polymorphism at the base number position 1018 of the glycoprotein Ibα gene; and

(22) a nucleic acid for analyzing polymorphism at the base number position 3932 of the apolipoprotein E gene.

[13] Fixed nucleic acids comprising the following two or more nucleic acid selected from the group consisting of the following (1) to (7) fixed to an insoluble support:

(1) a nucleic acid for analyzing polymorphism at the base number position 3932 of the apolipoprotein E gene;

(2) a nucleic acid for analyzing polymorphism at the base number position 1648 of the glycoprotein Ia gene;

(3) a nucleic acid for analyzing polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(4) a nucleic acid for analyzing polymorphism at the base number position 825 of G-protein β3 subunit gene;

(5) a nucleic acid for analyzing polymorphism at the base number position -482 of the apolipoprotein C-III gene; and

(6) a nucleic acid for analyzing polymorphism at the base number position -6 of the angiotensinogen gene.

[14] Fixed nucleic acids comprising the following two or more nucleic acid selected from the group consisting of the following (7) to (11) fixed to an insoluble support:

(7) a nucleic acid for analyzing polymorphism at the base number position 1186 of the thrombospondin 4 gene;

(8) a nucleic acid for analyzing polymorphism at the base number position -863 of the tumor necrosis factor-α gene;

(9) a nucleic acid for analyzing polymorphism at the base number position 2136 of the thrombomodulin gene;

(10) a nucleic acid for analyzing polymorphism at the base number position 5713 of the thrombopoietin gene; and

(11) a nucleic acid for analyzing polymorphism at the base number position 994 of the platelet-activating factor acetylhydrolase gene.

[15] Fixed nucleic acids comprising the following two or more nucleic acid selected from the group consisting of the following (12) to (17) fixed to an insoluble support:

(12) a nucleic acid for analyzing polymorphism at the base number position 561 of the E-selectin gene;

(13) a nucleic acid for analyzing polymorphism at the base number position 2445 of the fatty acid-binding protein 2 gene;

(14) a nucleic acid for analyzing polymorphism at the base number position 1018 of glycoprotein Ibα gene;

(15) a nucleic acid for analyzing polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(16) a nucleic acid for analyzing polymorphism at the base number position 584 of the paraoxonase gene; and

(17) a nucleic acid for analyzing polymorphism at the base number position 3932 of the apolipoprotein E gene.

[16] Fixed nucleic acids comprising the following two or more nucleic acid selected from the group consisting of the following (18) to (22) fixed to an insoluble support:

(18) a nucleic acid for analyzing polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene;

(19) a nucleic acid for analyzing polymorphism at the base number position -482 of the apolipoprotein C-III gene;

(20) a nucleic acid for analyzing polymorphism at the base number position 584 of the paraoxonase gene;

(21) a nucleic acid for analyzing polymorphism at the base number position 1018 of the glycoprotein Ibα gene; and

(22) a nucleic acid for analyzing polymorphism at the base number position 3932 of the apolipoprotein E gene.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a table summarizing 112 gene polymorphisms examined in a screening association study in Examples.

Figure 2 is also a table summarizing 112 gene polymorphisms examined in a screening association study in Examples.

Figure 3 is a table summarizing primers (SEQ ID NOs: 31, 32, 33, 28, 29, 30, 16, 17, 18, 46, 47, 48, 49, 50, 51, 25, 26, 27, 19, 20, 21, 52, 53, 54, 57, 58, 59, 55 and 56 in this order from the top), probes (SEQ ID NOs: 60, 61, 62, 63, 64, 65, 66 and 67 in this order from the top) and other conditions used to determine the genotype in Examples. In Figure 3, FITC denotes fluorescein isothiocyanate, TxR denotes Texas Red and Biotin denotes biotin, respectively.

Figure 4 is also a table summarizing primers (SEQ ID NOs: 43, 44, 45, 37, 38, 39, 40, 41, 42, 34, 35, 36, 22, 23 and 24 in this order from the top) and other conditions used to determine the genotype in Examples. In Figure 4, FITC denotes fluorescein isothiocyanate and TxR denotes Texas Red and Biotin denotes biotin, respectively.

Figure 5 is a table summarizing single nucleotide polymorphisms examined in an association study in Examples.

Figure 6 is a table summarizing the background data of 1620 lesions in men examined in an association study in Examples. Each data is represented by average ± standard deviation or percentage (%). In table, *1 denotes $P < 0.0001$ (versus corresponding “No restenosis”), *2 denotes $P < 0.001$ (versus corresponding “No

restenosis"), *3 denotes $P < 0.05$ (versus corresponding "No restenosis") and *4 denotes $P < 0.005$ (versus corresponding "No restenosis"), respectively.

Figure 7 is a table summarizing the background data of 771 lesions in women examined in an association study in Examples. Each data is represented by average ± standard deviation or percentage (%). In table, *1 denotes $P < 0.005$ (versus corresponding "No restenosis"), *2 denotes $P < 0.05$ (versus corresponding "No restenosis"), *3 denotes $P < 0.0001$ (versus corresponding "No restenosis") and *4 denotes $P < 0.001$ (versus corresponding "No restenosis"), respectively.

Figure 8 is a table summarizing gene polymorphisms and results of multivariate logistic regression analysis examined in the association study (in men).

Figure 9 is a table summarizing gene polymorphisms and results of multivariate logistic regression analysis examined in the association study (in women).

Figure 10 is a table showing results of step forward selection method of multivariate logistic regression analysis of gene polymorphisms associated with restenosis after coronary angioplasty (in men).

Figure 11 is a table showing results of step forward selection method of multivariate logistic regression analysis of gene polymorphisms associated with restenosis after coronary angioplasty (in women).

Figure 12 is a table showing results of diagnosis of genetic risk of restenosis after balloon dilatation using a combination of five gene polymorphisms in men.

Figure 13 is a table showing results of diagnosis of genetic risk of restenosis after stent implantation using a combination of five gene polymorphisms in men.

Figure 14 is a table showing results of diagnosis of genetic risk of restenosis after balloon dilatation using a combination of five gene polymorphisms in women.

Figure 15 is a table showing results of diagnosis of genetic risk of restenosis after stent implantation using a combination of five gene polymorphisms in women.

Figure 16 is a graph showing a correlation between the cumulative odds ratio for restenosis after coronary angioplasty and the number of single nucleotide polymorphisms. (○) shows the correlation in restenosis after balloon dilatation and (●) shows the correlation in restenosis after stent implantation, and (A) shows the correlation in men and (B) shows the correlation in women. In (A), SNPs of restenosis after balloon dilatation include: SNP 1: ApoE (3932T→C) polymorphism; SNP 2: GPIa (1648A→G) polymorphism; SNP 3: TNFα (-863C→A) polymorphism; SNP 4: G-protein β3 (825C→T) polymorphism; SNP5: ApoC-III

(-482C→T) polymorphism; and SNP6: AGT (-6G→A) polymorphism. Similarly, in (A) SNPs of restenosis after stent implantation include: SNP1: TSP4 (1186G→C) polymorphism, SNP2: TNFα (-863C→A) polymorphism; SNP3: TM (2136C→T) polymorphism; SNP4: TPO (5713A→G) polymorphism; and SNP5: PAF-AH (994G→T). In (B) SNPs of restenosis after balloon dilatation include: SNP 1: E-selectin gene (561A→C) polymorphism; SNP2: FABP2 (2445G→A) polymorphism; SNP3: GPIbα (1018C→T) polymorphism; SNP4: PAI1 (-668/4G→5G) polymorphism; SNP5: PON (584G→A) polymorphism; and SNP6: ApoE (3932T→CPAI1) polymorphism. Similarly, in (B) SNPs of restenosis after stent implantation include: SNP1: PAI1 (-668/4G→5G) polymorphism; SNP2: ApoC-III (-482C→T) polymorphism; SNP3: PON (584G→A) polymorphism; SNP4: GPIbα (1018C→T) polymorphism; and SNP5: ApoE (3932T→C) polymorphism.

BEST MODE FOR CARRYING OUT THE INVENTION

The first aspect of the present invention relates to a method of detecting the genotype in a nucleic acid sample. One embodiment of the present invention is featured by including the step of analyzing two or more polymorphisms selected from the group consisting of the following (1) to (6). Another embodiment is featured by including the step of analyzing two or more polymorphisms selected from the group consisting of the following (7) to (11). Further embodiment is featured by including the step of analyzing two or more polymorphisms selected from the group consisting of the following (12) to (17). Yet further embodiment is featured by including the step of analyzing two or more polymorphisms selected from the group consisting of the following (18) to (22). Note here that it is possible to determine, based on the information about polymorphism which was obtained in the above-mentioned step, the genotype of the nucleic acid sample, and thereby to assess, based on the genotype determined, a genetic risk of restenosis after coronary angioplasty.

(1) polymorphism at the base number position 3932 of the apolipoprotein E gene: 3932T→C (hereinafter, also referred to as "ApoE (3932T→C) polymorphism")

(2) polymorphism at the base number position 1648 of the glycoprotein Ia gene: 1648A→G (hereinafter, also referred to as "GPIa (1648A→G) polymorphism")

(3) polymorphism at the base number position -863 of the tumor necrosis factor-α gene: -863C→A (hereinafter, also referred to as "TNFα (-863C→A) polymorphism")

(4) polymorphism at the base number position 825 of the G-protein β3 subunit gene: 825C→T (hereinafter, also referred to as "G-protein β3 (825C→T) polymorphism")

(5) polymorphism at the base number position -482 of the apolipoprotein C-III gene: -482C→T (hereinafter, also referred to as "ApoC-III (-482C→T) polymorphism")

(6) polymorphism at the base number position -6 of the angiotensinogen gene: -6G→A (hereinafter, also referred to as "AGT (-6G→A) polymorphism")

(7) polymorphism at the base number position 1186 of the thrombospondin 4 gene: 1186G→C (hereinafter, also referred to as "TSP4 (1186G→C) polymorphism")

(8) polymorphism at the base number position -863 of the tumor necrosis factor-α gene: -863C→A (hereinafter, also referred to as "TNFα (-863C→A) polymorphism")

(9) polymorphism at the base number position 2136 of the thrombomodulin gene: 2136C→T (hereinafter, also referred to as "TM (2136C→T) polymorphism")

(10) polymorphism at the base number position 5713 of the thrombopoietin gene: 5713A→G (hereinafter, also referred to as "TPO (5713A→G) polymorphism")

(11) polymorphism at the base number position 994 of the platelet-activating factor acetylhydrolase gene: 994G→T (hereinafter, also referred to as "PAF-AH (994G→T) polymorphism")

(12) polymorphism at the base number position 561 of the E-selectin gene: 561A→C (hereinafter, also referred to as "E-selectin (561A→C) polymorphism")

(13) polymorphism at the base number position 2445 of the fatty acid-binding protein 2 gene: 2445G→A (hereinafter, also referred to as "FABP2 (2445G→A)polymorphism")

(14) polymorphism at the base number position 1018 of the glycoprotein Ibα gene: 1018 C→T (hereinafter, also referred to as "GPIbα (1018C→T) polymorphism")

(15) polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene: -668/4G→5G (hereinafter, also referred to as "PAI1

(-668/4G→5G) polymorphism")

(16) polymorphism at the base number position 584 of the paraoxonase gene: 584G→A (hereinafter, also referred to as "PON (584G→A) polymorphism")

(17) polymorphism at the base number position 3932 of the apolipoprotein E gene: 3932T→C (hereinafter, also referred to as "ApoE (3932T→C) polymorphism")

(18) polymorphism at the base number position -668 of the plasminogen activator inhibitor-1 gene: -668/4G→5G (hereinafter, also referred to as "PAI1 (-668/4G→5G) polymorphism")

(19) polymorphism at the base number position -482 of the apolipoprotein C-III gene: -482C→T (hereinafter, also referred to as "ApoC-III (-482C→T) polymorphism")

(20) polymorphism at the base number position 584 of the paraoxonase gene: 584G→A (hereinafter, also referred to as "PON (584G→A) polymorphism")

(21) polymorphism at the base number position 1018 of the glycoprotein Ibα gene: 1018C→T (hereinafter, also referred to as "GPIbα (1018C→T) polymorphism")

(22) polymorphism at the base number position 3932 of the apolipoprotein E gene: 3932T→C (hereinafter, also referred to as "ApoE (3932T→C) polymorphism")

In the above, description such as 3932T→C means that polymorphism at the relevant base number position consists of two genotypes, bases before and after the arrow. Herein, -668/4G→5G means a polymorphism consisting of a genotype having four G (guanines) existing successively in the 3' direction from the base number position -668 and a genotype having five G existing successively in the 3' direction from the base number position -668.

The base number of each gene is expressed using as standards the known sequences which are registered in the public database, GenBank (NCBI). Note here that in the base sequence of SEQ ID NO: 1 (Accession No. M10065 J03053 J03054: Human apolipoprotein E (epsilon-4 allele) gene, complete cds), the 3932nd base corresponds to the base at position 3932 of the apolipoprotein E gene. Similarly, in the base sequence of SEQ ID NO: 2 (Accession No. X17033 M28249: Human mRNA for integrin alpha-2 subunit), the 1648th base corresponds to the base

at position 1648 of the glycoprotein Iα gene; in the base sequence of SEQ ID NO: 3 (Accession No. L11698: Homo sapiens tumor necrosis factor alpha gene, promoter region), the 197th base corresponds to the base at position -863 of the tumor necrosis factor-α gene; in the base sequence of SEQ ID NO: 4 (Accession No. M31328: Human guanine nucleotide-binding protein beta-3 subunit mRNA, complete cds), the 831st base corresponds to the base at position 825 of the G-protein β3 subunit gene; in the base sequence of SEQ ID NO: 5 (Accession No. X13367: Human DNA for apolipoprotein C-III 5'-flank), the 936th base corresponds to the base at position -482 of the apolipoprotein C-III gene; in the sequence of SEQ ID NO: 6 (Accession No. X15323: H.sapiens angiotensinogen gene 5' region and exon 1), the 463rd base corresponds to the base at position -6 of the angiotensinogen gene; in the sequence of SEQ ID NO: 7 (Accession No. Z19585: H.sapiens mRNA for thrombospondin-4), the 1186th base corresponds to the base at position 1186 of the thrombospondin 4 gene; in the sequence of SEQ ID NO: 8 (Accession No. D00210: Homo sapiens gene for thrombomodulin precursor, complete cds), the 2136th base corresponds to the base at position 2136 of the thrombomodulin gene; in the sequence of SEQ ID NO: 9 (Accession No. L36051: Human thrombopoietin gene, complete cds), the 5753rd base corresponds to the base at position 5713; in the sequence of SEQ ID NO: 10 (Accession No. U20157: Human platelet-activating factor acetylhydrolase mRNA, complete cds), the 996th base corresponds to the base at position 994 of the platelet-activating factor acetylhydrolase gene; in the sequence of SEQ ID NO: 11 (Accession No. M24736: Human endothelial leukocyte adhesion molecule 1 (ELAM-1) mRNA, complete cds), the 561st base corresponds to the base at position 561 of the E-selectin gene; in the sequence of SEQ ID NO: 12 (Accession No. M18079 J03465: Human, intestinal fatty acid binding protein gene, complete cds, and an Alu repetitive element.), the 2445th base corresponds to the base at position 2445 of the fatty acid-binding protein 2 gene; in the sequence of SEQ ID NO: 13 (Accession No. J02940: Human platelet glycoprotein Ib alpha chain mRNA, complete cds), the 524th base corresponds to the base at position 1018 of the glycoprotein Ibα gene; in the sequence of SEQ ID NO: 14 (Accession No. X13323: Human gene for plasminogen activator inhibitor 1 (PAI-1) 5'-flank and exon 1), the 131st base corresponds to the base at position -668 of the plasminogen activator inhibitor-1 gene; and in the sequence of SEQ ID NO: 15 (Accession No. M63012: H.sapiens serum paraoxonase (PON) mRNA, complete cds), the 584th base corresponds to the base at position 584 of the paraoxonase gene.

In the present invention, "analyzing polymorphism" means the investigation as to what genotype a nucleic acid sample has in the gene polymorphism to be analyzed. It is the same meaning as the investigation on the base (base sequence) of the position in which the polymorphism exists. Typically, for example, in the case of the analysis of the ApoE (3932T→C) polymorphism, it refers to investigation on what genotype, i.e., CC (the base at position 3932 is a homozygote of allele C), TC (the base at position 3932 is a heterozygote of allele T and allele C) and TT (the base at position 3932 is a homozygote of allele T), the apolipoprotein E gene in a nucleic acid sample has.

As shown in Examples mentioned below, the polymorphisms mentioned (1) to (6) above are polymorphisms that are recognized as being particularly effective to be used in determining genetic risk of restenosis after coronary angioplasty in an analysis of Japanese male subjects who underwent balloon dilatation. Therefore, analysis targeting these polymorphisms enables diagnosis with higher accuracy and with higher predictability when balloon dilatation is performed as a coronary angioplasty and subjects are men (particularly, Japanese men).

Similarly, as shown in Examples mentioned below, the polymorphisms mentioned (7) to (11) above are polymorphisms that are recognized as being particularly effective to be used in determining genetic risk of restenosis after coronary angioplasty in an analysis of Japanese male subjects who underwent stent implantation. Therefore, analysis targeting these polymorphisms enables diagnosis with higher accuracy and with higher predictability when stent implantation is performed as a coronary angioplasty and subjects are men (particularly, Japanese men).

Similarly, as shown in Examples mentioned below, the polymorphisms mentioned (12) to (17) above are polymorphisms that are recognized as being particularly effective to be used in determining genetic risk of restenosis after coronary angioplasty in an analysis of Japanese female subjects who underwent balloon dilatation. Therefore, analysis targeting these polymorphisms enables diagnosis with higher accuracy and with higher predictability when balloon dilatation is performed as a coronary angioplasty and subjects are women (particularly, Japanese women).

Similarly, as shown in Examples mentioned below, the polymorphisms mentioned (18) to (22) above are polymorphisms that are recognized as being particularly effective to be used in determining genetic risk of restenosis after coronary angioplasty in an analysis of Japanese female subjects who underwent stent implantation. Therefore, analysis targeting these polymorphisms enables diagnosis with higher accuracy and with higher predictability when stent implantation is performed as a coronary angioplasty and subjects are women (particularly, Japanese women).

Herein, in principle, in proportion to the increase in the number of polymorphisms to be analyzed, the genotypes of nucleic acid sample are classified more finely. Thereby, it is possible to diagnose a genetic risk of restenosis after coronary angioplasty with higher predictability. From this viewpoint, it is preferable to detect the genotype by analyzing a larger number of polymorphisms in the above-mentioned polymorphisms (1) to (6). Therefore, it is the most preferable to analyze all of the polymorphisms (1) to (6). In the case where detection is carried out by combining five or less of polymorphisms, it is preferable to preferentially select the polymorphisms with higher odds ratios as in Examples mentioned below. For example, in the case where five polymorphisms are used in combination, it is preferable to select five polymorphisms with higher odds ratio, that is, to select (1), (2), (3), (4) and (5). Similarly, in the case where four polymorphisms are used in combination, it is preferable to select (1), (3), (4) and (5). Similarly, in the case where three polymorphisms are used in combination, it is preferable to select (1), (3) and (4).

Similarly, in the case where two or more polymorphisms selected from the group consisting of polymorphisms (7) to (11), it is most preferable to analyze all these polymorphisms, that is, five polymorphisms. In the case where detection is carried out by combining four or less of polymorphisms, it is preferable to preferentially select the polymorphisms with higher odds ratios in Examples mentioned below. For example, in the case where four polymorphisms are used in combination, it is preferable to select four polymorphisms with higher odds ratio, that is, to select (7), (8), (9) and (10). Similarly, in the case where three polymorphisms are used in combination, it is preferable to select (7), (8) and (9). Similarly, in the case where two polymorphisms are used in combination, it is

preferable to select (7) and (8).

Similarly, in the case where two or more polymorphisms selected from the group consisting of polymorphisms (12) to (17), it is most preferable to analyze all these polymorphisms, that is, six polymorphisms. In the case where detection of the genotype is carried out by combining five or less of polymorphisms, it is preferable to preferentially select the polymorphisms with higher odds ratios in Examples mentioned below. For example, in the case where five polymorphisms are used in combination, it is preferable to select five polymorphisms with higher odds ratio, that is, to select (12), (13), (14), (15) and (16). Similarly, in the case where four polymorphisms are used in combination, it is preferable to select (12), (13) (14) and (15). Similarly, in the case where three polymorphisms are used in combination, it is preferable to select (12), (13) and (14).

Similarly, in the case where two or more polymorphisms selected from the group consisting of polymorphisms (18) to (22), it is most preferable to analyze all these polymorphisms, that is, five polymorphisms. In the case where detection of the genotype is carried out by combining four or less of polymorphisms, it is preferable to preferentially select the polymorphisms with higher odds ratios in Examples mentioned below. For example, in the case where four polymorphisms are used in combination, it is preferable to select four polymorphisms with higher odds ratio, that is, to select (18), (19), (20) and (21). Similarly, in the case where three polymorphisms are used in combination, it is preferable to select (18), (19) and (20). Similarly, in the case where two polymorphisms are used in combination, it is preferable to select (18) and (19).

A method for analyzing each genetic polymorphism is not particularly limited. Known methods may include, for example, amplification by PCR using an allele-specific primer (and probe), a method for analyzing polymorphism of amplified product by means of fluorescence or luminescence, PCR-RFLP (polymerase chain reaction–restriction fragment length polymorphism) method, PCR-SSCP (polymerase chain reaction–single strand conformation polymorphism) method (Orita, M. et al., Proc. Natl. Acad. Sci., U.S.A., 86, 2766-2770 (1989), etc.), PCR-SSO (specific sequence oligonucleotide) method, which use PCR method, ASO (allele specific oligonucleotide) hybridization method combining the

PCR-SSO method and a dot hybridization method (Saiki, Nature, 324, 163-166 (1986), etc.), or TaqMan-PCR method (Livak, KJ, Genet Anal, 14, 143 (1999), Morris, T. et al., J. Clin. Microbiol.,34, 2933 (1996)), Invader method (Lyamichev V et al., Nat Biotechnol, 17, 292 (1999)), MALDI-TOF/MS (matrix) method using a primer extension method (Haff LA, Smirnov IP, Genome Res 7, 378 (1997)), RCA (rolling cycle amplification) method (Lizardi PM et al., Nat Genet 19,225 (1998)), a method using DNA microchip or micro-array (Wang DG et al., Science 280, 1077 (1998), etc.)), a primer extension method, a Southern blot hybridization method, a dot hybridization method (Southern, E., J. Mol. Biol. 98, 503-517 (1975)), etc.), or the like. Furthermore, an analysis may be made by direct sequencing of the portion of polymorphism which is subject to analysis. Note here that polymorphisms may be analyzed by combining these methods ad libitum.

In the case where the amount of nucleic acid sample is small, it is preferable to analyze it by a method using PCR (for example, PCR-RFLP method) from the viewpoint of detection sensitivity or accuracy. Furthermore, any of the above-mentioned analysis methods may be employed after nucleic acid sample is amplified in advance (including a partial region of nucleic acid sample) by a gene amplification such as PCR method or a method applying PCR method.

Meanwhile, in the case where a large number of nucleic acid samples are analyzed, a method capable of analyzing a large number of samples in a relatively short period of time, for example, allele-specific PCR method, allele-specific hybridization method, TaqMan-PCR method, Invader method, MALDI-TOF/MS (matrix) method using a primary extension method, RCA (rolling cycle amplification) method, or a method using a DNA chip or a micro-array.

The above methods use nucleic acids (also called "nucleic acids for analyzing polymorphism" in the present invention), e.g., primer and probe, in accordance with each method. An example of the nucleic acids for analyzing polymorphism may include a nucleic acid having a sequence complementary to a given region including the site of polymorphism (partial DNA region) in a gene which contains polymorphism to be analyzed, and a nucleic acid (primer) which has a sequence complementary to a given region including the site of polymorphism (partial DNA region) in a gene which contains polymorphism to be analyzed and which is designed to specifically amplify the DNA fragment containing the relevant

site of polymorphism. In the case where polymorphism at position 3932 of the apolipoprotein E gene is a subject to be analyzed, an example of such nucleic acids includes a nucleic acid having a sequence complementary to a partial DNA region including the position 3932 of the apolipoprotein E gene whose base at position 3932 is T (thymine), or a nucleic acid having a sequence complementary to a partial DNA region including the position 3932 of the apolipoprotein E gene whose base at position 3932 is C (cytosine).

Other concrete examples of nucleic acids for analyzing polymorphism may include a set of nucleic acids which is designed to specifically amplify the partial DNA region that contains the relevant site of polymorphism only in the case where the site of polymorphism to be analyzed is a certain genotype. A more concrete example may include a set of nucleic acids which is designed to specifically amplify the partial DNA region including the site of polymorphism to be analyzed and which consists of a sense primer that specifically hybridizes the partial DNA region including the relevant site of polymorphism in an antisense strand whose site of polymorphism is a certain genotype and of an antisense primer that specifically hybridizes a partial region in the sense strand. In the case where polymorphism at position 3932 of the apolipoprotein E gene is a subject to the analysis, examples of such a set of nucleic acids include a set of nucleic acids which is designed to specifically amplify the partial DNA region including the base at position 3932 of the apolipoprotein E gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at 3932 is T (thymine) and of an antisense primer that specifically hybridizes a partial region in the sense strand; or a set of nucleic acids which consists of a sense primer that specifically hybridizes the partial DNA region including the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at position 3932 is C (cytosine) and of an antisense primer that specifically hybridizes a partial region in the sense strand. The length of the partial DNA region to be amplified here is set accordingly in a range which is appropriate for its detection, and is for example, 50 bp to 200 bp, and preferably 80 bp to 150 bp. A more concrete example may include a set of nucleic acids for analyzing the ApoE (3932T→C) polymorphism containing the following sequences. Note here that an underlined part in the following sequences means a part corresponding to the polymorphism.

Furthermore, in the sequence, N denotes any of A, T, C and G.

antisense primer

GGACATGGAGGACGTNCG: SEQ ID NO: 16, or

CGGACATGGAGGACGTNTG: SEQ ID NO: 17

sense primer

CGCGGTACTGCACCAGGC: SEQ ID NO: 18

Similarly, an example of a nucleic acid primer for analyzing the GPIa (1648A→G) polymorphism may include a set containing the following sequences.

sense primer

GAGTCTACCTGTTTACTATCAANAA: SEQ ID NO: 19, or

GAGTCTACCTGTTTACTATCAANGA: SEQ ID NO: 20

antisense primer

ACCAGTACTAAAGCAAATTAAACT: SEQ ID NO: 21

Similarly, an example of a nucleic acid primer for analyzing the TNFα (-863C→A) polymorphism may include a set containing the following sequences.

antisense primer

GGCCCTGTCTTCGTTAANGG: SEQ ID NO: 22, or

ATGGCCCTGTCTTCGTTAANTG: SEQ ID NO: 23

sense primer

CCAGGGCTATGGAAGTCGAGTATC: SEQ ID NO: 24

Similarly, an example of a nucleic acid primer for analyzing the G-protein β3 (825C→T) polymorphism may include a set containing the following sequences.

sense primer

TCTGCGGCATCACGTNCG: SEQ ID NO: 25, or

TCTGCGGCATCACGTNTG: SEQ ID NO: 26

antisense primer

GAATAGTAGGCGGCCACTGA: SEQ ID NO: 27

Similarly, an example of a nucleic acid primer for analyzing the ApoC-III (-482C→T) polymorphism may include a set containing the following sequences.

sense primer

CGGAGCCACTGATGCNCG: SEQ ID NO: 28, or

CGGAGCCACTGATGCNTG: SEQ ID NO: 29
antisense primer
TGTTTGGAGTAAAGGCACAGAA: SEQ ID NO: 30

Similarly, an example of a nucleic acid primer for analyzing the AGT (-6G→A) polymorphism may include a set containing the following sequences.

antisense primer
CGGCAGCTTCTTCCCNCG: SEQ ID NO: 31, or
CGGCAGCTTCTTCCCNTG: SEQ ID NO: 32
sense primer
CCACCCCTCAGCTATAAATAGG: SEQ ID NO: 33

Similarly, an example of a nucleic acid primer for analyzing the TSP4 (1186G→C) polymorphism may include a set containing the following sequences.

sense primer
CGAGTTGGGAACGCACNCT: SEQ ID NO: 34, or
CGAGTTGGGAACGCACNGT: SEQ ID NO: 35
antisense primer
GGTCTGCACTGACATTGATGAG: SEQ ID NO: 36

Similarly, an example of a nucleic acid primer for analyzing the TM (2136C→T) polymorphism may include a set containing the following sequences.

sense primer
CCCGACTCGGCCCTTNCC: SEQ ID NO: 37, or
CCCGACTCGGCCCTTNTC: SEQ ID NO: 38
antisense primer
GTCACAGTCGGTGCCAATGT: SEQ ID NO: 39

Similarly, an example of a nucleic acid primer for analyzing the TPO (5713A→G) polymorphism may include a set containing the following sequences.

sense primer
CCGACATCAGCATTGTCTNAT: SEQ ID NO: 40, or
CCGACATCAGCATTGTCTNGT: SEQ ID NO: 41
antisense primer
CTGCAGGGAAGGGAGCTGT: SEQ ID NO: 42

Similarly, an example of a nucleic acid primer for analyzing the PAF-AH (994G→T) polymorphism may include a set containing the following sequences.

sense primer

TTCTTTTGGTGGAGCAACNGT: SEQ ID NO: 43, or

ATTCTTTTGGTGGAGCAACNTT: SEQ ID NO: 44

antisense primer

TCTTACCTGAATCTCTGATCTTCA: SEQ ID NO: 45

Similarly, an example of a nucleic acid primer for analyzing the E-selectin (561A→C) polymorphism may include a set containing the following sequences.

antisense primer

ACATTCACCGTGGCCANTG: SEQ ID NO: 46, or

CATTCACCGTGGCCANGG: SEQ ID NO: 47

sense primer

AGCTGCCTGTACCAATACATCC: SEQ ID NO: 48

Similarly, an example of a nucleic acid primer for analyzing the FABP2 (2445G→A) polymorphism may include a set containing the following sequences.

sense primer

TCACAGTCAAAGAATCAAGNGC: SEQ ID NO: 49, or

ATTCACAGTCAAAGAATCAAGNAC: SEQ ID NO: 50

antisense primer

CAAAAACAACTTCAATGTTTCGA: SEQ ID NO: 51

Similarly, an example of a nucleic acid primer for analyzing the GPIbα (1018C→T) polymorphism may include a set containing the following sequences.

sense primer

CCCAGGGCTCCTGNCG: SEQ ID NO: 52, or

CCCCAGGGCTCCTGNTG: SEQ ID NO: 53

antisense primer

TGAGCTTCTCCAGCTTGGGTG: SEQ ID NO: 54

Similarly, an example of a nucleic acid primer for analyzing the PAI1 (-668/4G→5G) polymorphism may include a set containing the following

sequences.

sense primer

GGCACAGAGAGAGTCTGGACACG: SEQ ID NO: 55

antisense primer

GGCCGCCTCCGATGATACA: SEQ ID NO: 56

Similarly, an example of a nucleic acid primer for analyzing the PON (584G→A) polymorphism may include a set containing the following sequences.

sense primer

ACCCAAATACATCTCCCAGGANCG: SEQ ID NO: 57, or

AACCCAAATACATCTCCCAGGNCT: SEQ ID NO: 58

antisense primer

GAATGATATTGTTGCTGTGGGAC: SEQ ID NO: 59

On the other hand, a concrete example of the probe can include:

as a probe for analyzing Apo C-III (-482C→T) polymorphism,

AGCCACTGATGCNCGGTCT: SEQ ID NO: 60, or

AGCCACTGATGCNTGGTCT: SEQ ID NO: 61,

as a probe for analyzing E-selectin (561A→C) polymorphism,

CACCGTGGCCANTGCAGGAT: SEQ ID NO: 62, or

CACCGTGGCCANGGCAGGAT: SEQ ID NO: 63,

as a probe for analyzing FABP2 (2445G→A) polymorphism,

GAATCAAGNGCTTTTCGAAACATT: SEQ ID NO: 64, or

GAATCAAGNACTTTTCGAAACATT: SEQ ID NO: 65,

as a probe for analyzing PAI1 (-668/4G→5G) polymorphism,

TGGACACGTGGGGGAGTCAG: SEQ ID NO: 66, or

TGGACACGTGGGGAGTCAGC: SEQ ID NO: 67.

The above nucleic acid primers and nucleic acid probes are mere examples. Nucleic acid primers may contain a partially modified base sequence as long as they can carry out the aimed amplification reaction without inconvenience, while nucleic acid probes may contain a partially modified base sequence as long as they can carry

out the aimed hybridization reaction without inconvenience. "Partially modified" herein means that a part of bases is deleted, replaced, inserted, and/or added. The numbers of modified bases are for example one to seven, preferably one to five, and more preferably one to three. Note here that such a modification is made in the portions other than bases which correspond to the site of polymorphism, in principle. However, in the case where the polymorphism to be analyzed is PAI1 (-668/4G→5G) polymorphism, it is also possible to use a primer or probe obtained by modifying a part of the base corresponding to the polymorphism site.

As nucleic acids for analyzing polymorphism (probes or primers), DNA fragments or RNA fragments are used accordingly in response to the analysis method employed. The base length of nucleic acids for analyzing polymorphism may be sufficient if it exerts respective functions of each nucleic acid. Base lengths in the case of use as primers are for example, 10 to 50 bp, preferably 15 to 40 bp, and more preferably 15 to 30 bp.

Note here that in the case of use as primers, some mismatches to the sequence which constitutes the template may be admitted as long as the primer can specifically hybridize the subject for amplification and amplify the target DNA fragment. In the case of probes, some mismatches to the sequence which is subject to detection may be similarly admitted as long as the probe can specifically hybridize the sequence which is subject to detection. The numbers of mismatches are one to several, preferably one to five, and more preferably one to three.

Nucleic acids for analyzing polymorphism (primers and probes) can be synthesized in accordance with known methods, e.g., phosphodiester method. Note here that textbooks (e.g., Molecular Cloning, Third Edition, Cold Spring Harbor Laboratory Press, New York) can be referred with respect to the design, synthesis, and others of nucleic acids for analyzing polymorphism.

Nucleic acids for analyzing polymorphism in the present invention can be labeled with labeling substances in advance. The use of such labeled nucleic acids allows, for example, the analysis of polymorphism by using the labeling amount in the product of amplification as a marker. Furthermore, by labeling two kinds of primers which were designed specifically amplify the partial DNA region in the gene of each genotype that constitute polymorphism with labeling substances that are different from each other, the genotype of a nucleic acid sample can be

discriminated according to the labeling substance and labeling amount to be detected based on the product of amplification. Concrete examples of detection methods using these labeled primers may include: a method for detecting polymorphism, comprising labeling, with fluorescein isocyanate and Texas red, two kinds of nucleic acid primers (allele-specific sense primers) that respectively and specifically hybridize the sense strand of each genotype constituting polymorphism; amplifying the partial DNA region including the site of polymorphism by using these labeled primers and the antisense primers that specifically hybridize the antisense strand; and measuring the labeling amount of each fluorescent substance in the product of amplification obtained. Note here that labeling of the antisense primer herein with for example, biotin allows the separation of the product of amplification by utilizing the specific binding between biotin and avidin.

Radioactive isotopes, for example, ^{32}P, and fluorescent substance, for example, fluorescein isothiocyanate, tetramethylrhodamine isothiocyanate, and Texas red, etc. can be exemplified as labeling substances to be used in labeling nucleic acids for analyzing polymorphism. The 5' terminal labeling method using alkaline phosphatase and T4 polynucleotide kinase, the 3' terminal labeling method using T4 DNA polymerase and Klenow fragment, nicktranslation method, random primer method (Molecular Cloning, Third Edition, Chapter 9, Cold Spring Harbor Laboratory Press, New York), and the like can be exemplified as labeling methods.

The above-mentioned nucleic acids for analyzing polymorphism can be used also under a condition fixed to an insoluble support. Processing of an insoluble support to be used for the fixation to several forms such as chips and beads allows the more simplified analysis of polymorphism by using these fixed nucleic acids.

A nucleic acid sample can be prepared from blood, skin cells, mucous cells, hair, and others from the subject according to known extraction methods and purification methods. In the case of including the gene which is subject to the analysis of polymorphism, the genome DNA of arbitrary length can be used as a nucleic acid sample. Furthermore, it is not necessary to use a nucleic acid sample in which all genes subject to the analysis are present on one nucleic acid. That is to say, as a nucleic acid sample of the present invention, both material in which all genes subject to the analysis are present on one nucleic acid and material in which

genes subject to the analysis are present separately on two or more nucleic acids can be used. Note here that material in a fragmented or partial condition may be accepted as long as the site of polymorphism to be analyzed is at least present, although genes subject to the analysis in a nucleic acid sample are not in a complete condition (i.e., a condition in which the full length of the gene is present).

Analysis of each gene polymorphism is carried out each by each of the gene polymorphism or a plurality or entire gene polymorphisms are carried out simultaneously. In the former case, for example, nucleic acid sample collected from the subjects is divided in accordance with the number of polymorphisms to be analyzed, and analysis of polymorphism is carried out individually. In the latter case, for example, analysis of polymorphism can be carried out by DNA chip or micro-array. Note here that "simultaneousness" herein not only imply that all operations of the analysis process are conducted simultaneously but also include the case in which part of operations (e.g., operation to amplify nucleic acid, hybridization or detection of the probe) is conducted simultaneously.

Polymorphism of each gene can be analyzed by using mRNA which is a product of transcription of the gene which is subject to the analysis. After extracting and purifying mRNA of the gene which is subject to the analysis from blood, urine, and others of the subject, for example, polymorphism can be analyzed with mRNA as a starting material by conducting methods, e.g., Northern blotting method (Molecular Cloning, Third Edition, 7.42, Cold Spring Harbor Laboratory Press, New York), dot blotting method (Molecular Cloning, Third Edition, 7.46, Cold Spring Harbor Laboratory Press, New York), RT-PCR method (Molecular Cloning, Third Edition, 8.46, Cold Spring Harbor Laboratory Press, New York), and methods using the DNA chip (DNA array), and the like.

In addition, in the above-mentioned polymorphism, polymorphism involved with changes in amino acids can analyzed by using the expression product of gene that is a subject to analysis. In this case, material, even being partial protein or partial peptide, can be used as a sample for analysis as long as it contains amino acids which correspond to the site of polymorphism.

Analysis methods using these expression products of gene may include: a

method for directly analyzing amino acids at the site of polymorphism, a method for immunologically analyzing utilizing changes of three-dimensional structure, or the like. As the former, a well-known amino acid sequence analysis method (a method using Edman method) can be used. As the latter, ELISA (enzyme-linked immunosorbent assay) using the monoclonal antibody or polyclonal antibody which has binding activity specific to the expression product of gene which has any of genotypes that constitute polymorphism; radioimmunoassay, immunoprecipitation method, immunodiffusion method, and the like can be used.

Information about polymorphisms to be obtained by conducting the detection methods of the present invention described above can be used to diagnose a genetic risk of restenosis after coronary angioplasty. That is to say, the present invention also provides a method for diagnosing a genetic risk of restenosis after coronary angioplasty, which comprises a step of determining the genotype in a nucleic acid sample based on information about polymorphism that was obtained by the above-detection methods, and a step of assessing a genetic risk of restenosis after coronary angioplasty based on the determined genotype of the nucleic acid sample. Herein, the determination of the genotype is typically to determine which genotype both alleles of nucleic acid samples have with respect to the polymorphism to be detected. In the case where the subject to be detected is, for example, ApoE (3932T→C) polymorphism, the detection of genotype is typically, an investigation on what genotype that is, TT (the base at position 3932 is a homozygote of allele T), CT (the base at position 3932 is a heterozygote of allele T and allele C) or CC (the base at position 3932 is a homozygote of allele C), the apolipoprotein E gene has in a nucleic acid sample.

By considering the results obtained in Example mentioned below, in order to enable a diagnosis of genetic risk of restenosis after coronary angioplasty with high accuracy and high predictability, for example, in the case of the ApoE (3932T→C) polymorphism, it is determined whether the genotype in a nucleic acid sample is CC or TC, or TT. Similarly, in the case of the GPIa (1648A→G) polymorphism, it is determined whether the genotype is GG, or AG or AA; in the case of the TNFα (-863C→A) polymorphism, it is determined whether the genotype is AA or CA, or CC; in the case of the G-protein β3 (825C→T) polymorphism, it is determined whether the genotype is TT, or CT or CC; in the case of the ApoC-III (-482C→T)

polymorphism, it is determined whether the genotype is TT or CT, or CC, or TT, or CT or CC; in the case of AGT (-6G→A) polymorphism, it is determined whether the genotype is AA or GA, or GG; in the case of TSP4 (1186G→C) polymorphism, it is determined whether the genotype is CC or GC, or GG; in the case of TM (2136C→T) polymorphism, it is determined whether the genotype is TT or CT, or CC; in the case of the TPO (5713A→G) polymorphism, it is determined whether the genotype is GG, or AG or AA; in the case of the PAF-AH (994G→T) polymorphism, it is determined whether the genotype is TT or GT, or GG; in the case of the E-selectin (561A→C) polymorphism, it is determined whether the genotype is CC or AC, or AA; in the case of glycoprotein FABP2 (2445G→A) polymorphism, it is determined whether the genotype is AA or GA, or GG; in the case of paraoxonase GPIbα (1018C→T) polymorphism, it is determined whether the genotype is TT or CT, or CC; in the case of PAI1 (-668/4G→5G) polymorphism, it is determined whether the genotype is 5G/5G or 4G/5G, or 4G/4G; in the case of PON (584G→A) polymorphism, it is determined whether the genotype is AA or GA, or GG.

Diagnosis of a genetic risk of restenosis after coronary angioplasty enables prediction of potentiality (susceptibility to development) in that restenosis might be developed after coronary angioplasty. That is to say, according to the diagnosis method of the present invention, it is possible to evaluate the risk of development of restenosis after coronary angioplasty. It is clinically significant that such an evaluation enables the selection of an appropriate treatment method in advance.

By utilizing the genetic information associated with the development of restenosis obtained by the present invention, it is possible to reduce the development rate of restenosis after coronary angioplasty. For example, as a result of carrying out the diagnostic method of the present invention, when the polymorphism to be analyzed is a genotype to increase the risk of development of restenosis, by introducing a gene having a genotype with low risk of development into a living body, due to the expression of the gene, the reduction of disease can be expected. The same treatment effect can be expected by a method including of introducing antisense strand with respect to mRNA of gene having a genotype with high risk of development of restenosis and suppressing the expression of the mRNA.

The introduction of such genes into the living body can be carried out by a

method, for example, a method using a plasmid for gene introduction or a virus vector, electroporation (Potter,H. et al., Proc. Natl. Acad. Sci. U.S.A. 81, 7161-7165(1984), an ultrasonic microbubble (Lawrie, A., et al. Gene Therapy 7, 2023-2027 (2000)), lipofection (Felgner, P.L. et al., Proc. Natl. Acad. Sci. U.S.A. 84, 7413-7417 (1984)), microinjection (Graessmann, M. & Graessmann, A., Proc. Natl. Acad. Sci. U.S.A. 73,366-370(1976)), and the like. By utilizing these methods, desired genes, etc. can be directly introduced (in vivo method) or indirectly introduced (ex vivo method).

Furthermore, by using an instrument such as a stent previously coated with gene, etc. (a gene held by a plasmid for introducing genes or virus vector may be coated), the above-mentioned gene introduction can be carried out at the same time or after the coronary angioplasty.

The second aspect of the present invention provides a kit to be used in the above-mentioned detecting method or diagnostic method in the present invention (a kit for detecting the genotype or a kit for diagnosing restenosis after coronary angioplasty). Such a kit contains nucleic acids (nucleic acids for analyzing polymorphism) for analyzing two or more polymorphisms selected from the group consisting of polymorphisms described in (1) to (6) above. As another embodiment, such a kit is constructed, which contains nucleic acids (nucleic acids for analyzing polymorphism) for analyzing two or more polymorphisms selected from the group consisting of polymorphisms described in (7) to (11) above. As a further embodiment, such a kit is constructed, which contains nucleic acids (nucleic acids for analyzing polymorphism) for analyzing two or more polymorphisms selected from the group consisting of polymorphisms described in (12) to (17) above. As a yet further embodiment, such a kit is constructed, which contains nucleic acids (nucleic acids for analyzing polymorphism) for analyzing two or more polymorphisms selected from the group consisting of polymorphisms described in (18) to (22) above.

In the analysis methods by which it is applied (a method which utilizes PCR using the above-mentioned allele-specific nucleic acids and the like, PCR-RFLP method, PCR-SSCP method, TaqMan-PCR method, Invader method, etc.), nucleic acids for analyzing polymorphism are designed as materials which can specifically amplifies (primer) or specifically detect (probe) the DNA region containing the polymorphism portion to be analyzed or mRNA which corresponds to the region.

Concrete examples of kits to be provided according to the present invention are described below.

A kit for detecting the genotype, comprising two or more nucleic acids selected from the group consisting of the following (1) to (6):

(1) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is T, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is C:

(2) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose base at position 1648 is A, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose base at position 1648 is G:

(3) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose base at position -863 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose base at position -863 is A:

(4) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene whose base at position 825 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene whose base at position 825 is T:

(5) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene whose base at position -482 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene whose base at position -482 is T: and

(6) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -6 of the angiotensinogen gene whose base at position -6 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -6 of the angiotensinogen gene whose base at position -6 is A.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (1) to (6). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (1) to (6) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (1) to (5) (a set of nucleic acids for analyzing polymorphisms with five highest odds ratio and P values which are considered in Example mentioned below), or kits may be constructed by selecting two or more nucleic acids from the group consisting of (1), (3), (4) and (5) (nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more nucleic acids selected from the group consisting of the following (7) to (11):

(7) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene whose base at position 1186 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene whose base at position 1186 is C;

(8) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose base at position -863 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose base at position -863 is A;

(9) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 2136 of the thrombomodulin gene whose base at position 2136 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 2136 of the thrombomodulin gene whose base at position 2136 is T;

(10) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 5713 of the thrombopoietin gene whose base at position 5713 is A, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 5713 of the thrombopoietin gene whose base at position 5713 is G; and

(11) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 994 of the platelet-activating factor

acetylhydrolase gene whose base at position 994 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 994 of the platelet-activating factor acetylhydrolase gene whose base at position 994 is T.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (7) to (11). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (7) to (11) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (7) to (10) (nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more nucleic acids from the group consisting of (7) to (9) (nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more nucleic acids selected from the group consisting of the following (12) to (17):

(12) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 561 of the E-selectin gene whose base at position 561 is A, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 561 of the E-selectin gene whose base at position 561 is C;

(13) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene whose base at position 2445 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene whose base at position 2445 is A;

(14) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is T;

(15) a nucleic acid having a sequence which is complementary to the partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene in which four G successively exist in the 3' direction from the position -668, or a

nucleic acid having a sequence which is complementary to the partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene in which five G successively exist in the 3' direction from the position -668;

(16) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is A; and

(17) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is T, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is C.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (12) to (17). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (12) to (17) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (12) to (16) (nucleic acids for analyzing polymorphisms with five highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more nucleic acids from the group consisting of (12) to (15) (nucleic acids with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more nucleic acids selected from the group consisting of the following (18) to (22):

(18) a nucleic acid having a sequence which is complementary to the partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene in which four G successively exist in the 3' direction from the position -668, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene in which five G successively exist in the 3' direction from the position -668;

(19) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene whose base at position -482 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position -482 of the

apolipoprotein C-III gene whose base at position -482 is T;

(20) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is G, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is A;

(21) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is C, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is T; and

(22) a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is T, or a nucleic acid having a sequence which is complementary to the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is C.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (18) to (22). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (18) to (22) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (18) to (21) (nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more nucleic acids from the group consisting of (18) to (20) (nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (1) to (6):

(1) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene only in the case where the base at position 3932 of the apolipoprotein E gene in a nucleic acid sample is T, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene only in the case where the base at position 3932 of the apolipoprotein E gene in

a nucleic acid sample is C;

(2) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene only in the case where the base at position 1648 of the glycoprotein Ia gene in a nucleic acid sample is A, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene only in the case where the base at position 1648 of the glycoprotein Ia gene in a nucleic acid sample is G;

(3) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene only in the case where the base at position -863 of the tumor necrosis factor-α gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene only in the case where the base at position -863 of the glycoprotein Ia gene in a nucleic acid sample is A;

(4) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene only in the case where the base at position 825 of the G-protein β3 subunit gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene only in the case where the base at position 825 of the G-protein β3 subunit gene in a nucleic acid sample is T;

(5) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene only in the case where the base at position -482 of the apolipoprotein C-III gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene only in the case where the base at position -482 of the apolipoprotein C-III gene in a nucleic acid sample is T; and

(6) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -6 of the angiotensinogen gene only in the case where the base at position -6 of the angiotensinogen gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -6 of the angiotensinogen gene only in the case where the base at position -6 of the angiotensinogen gene in a

nucleic acid sample is A.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (1) to (6). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (1) to (6) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (1) to (5) (sets of nucleic acids for analyzing polymorphisms with five highest odds ratio and *P* values which are considered in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (1), (3), (4) and (5) (nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (7) to (11):

(7) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene only in the case where the base at position 1186 of the thrombospondin 4 gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene only in the case where the base at position 1186 of the thrombospondin 4 gene in a nucleic acid sample is C;

(8) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene only in the case where the base at position -863 of the tumor necrosis factor-α gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene only in the case where the base at position -863 of the tumor necrosis factor-α gene in a nucleic acid sample is A;

(9) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 2136 of the thrombomodulin gene only in the case where the base at position 2136 of the thrombomodulin gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 2136 of the thrombomodulin gene only in the case where the base at position 2136 of the thrombomodulin gene in a nucleic acid sample is T;

(10) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 5713 of the thrombopoietin gene only in the case where the base at position 5713 of the thrombopoietin gene in a nucleic acid sample is A, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 5713 of the thrombopoietin gene only in the case where the base at position 5713 of the thrombopoietin gene in a nucleic acid sample is G; and

(11) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 994 of the platelet-activating factor acetylhydrolase gene only in the case where the base at position 994 of the platelet-activating factor acetylhydrolase gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 994 of the platelet-activating factor acetylhydrolase gene only in the case where the base at position 994 of the platelet-activating factor acetylhydrolase gene in a nucleic acid sample is T.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (7) to (11). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (7) to (11) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (7) to (10) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratio in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (7) to (9) (sets of nucleic acids for analyzing polymorphisms with three highest odds ratio in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (12) to (17):

(12) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 561 of the E-selectin gene only in the case where the base at position 561 of the E-selectin gene in a nucleic acid sample is A, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 561 of the E-selectin gene only in the case where the base at position 561 of the E-selectin gene in a nucleic acid sample is C;

(13) a set of nucleic acids which is designed to specifically amplify the partial

DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene only in the case where the base at position 2445 of the fatty acid-binding protein 2 gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene only in the case where the base at position 2445 of the fatty acid-binding protein 2 gene in a nucleic acid sample is A;

(14) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene only in the case where the base at position 1018 of the glycoprotein Ibα gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene only in the case where the base at position 1018 of the glycoprotein Ibα gene in a nucleic acid sample is T;

(15) a set of nucleic acids which is designed to specifically amplify a partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene only in the case where four G successively exist in the 3' direction from the position -668 in the plasminogen activator inhibitor-1 gene in a nucleic sample, or a set of nucleic acids which is designed to specifically amplify a partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene only in the case where five G successively exist in the 3' direction from the position -668 in the plasminogen activator inhibitor-1 gene in a nucleic sample;

(16) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 584 of the paraoxonase gene only in the case where the base at position 584 of the paraoxonase gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 584 of the paraoxonase gene only in the case where the base at position 584 of the paraoxonase gene in a nucleic acid sample is A; and

(17) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene only in the case where the base at position 3932 of the apolipoprotein E gene in a nucleic acid sample is T, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene only in the case where the base at position 3932 of the apolipoprotein E gene in

a nucleic acid sample is C.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (12) to (17). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (12) to (17) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (12) to (16) (sets of nucleic acids for analyzing polymorphisms with five highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (12) to (15) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (18) to (22):

(18) a set of nucleic acids which is designed to specifically amplify a partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene only in the case where four G successively exist in the 3' direction from the position -668 in the plasminogen activator inhibitor-1 gene in a nucleic sample, or a set of nucleic acids which is designed to specifically amplify a partial DNA region containing the part of sequence of the plasminogen activator inhibitor-1 gene only in the case where five G successively exist in the 3' direction from the position -668 in the plasminogen activator inhibitor-1 gene in a nucleic sample;

(19) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene only in the case where the base at position -482 of the apolipoprotein C-III gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene only in the case where the base at position -482 of the apolipoprotein C-III gene in a nucleic acid sample is T;

(20) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 584 of the paraoxonase gene only in the case where the base at position 584 of the paraoxonase gene in a nucleic acid sample is G, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 584 of the paraoxonase gene only in the case where the base at position 584 of the paraoxonase gene in a nucleic acid sample

is A;

(21) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene only in the case where the base at position 1018 of the glycoprotein Ibα gene in a nucleic acid sample is C, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene only in the case where the base at position 1018 of the glycoprotein Ibα gene in a nucleic acid sample is T; and

(22) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene only in the case where the base at position 3932 of the apolipoprotein E gene in a nucleic acid sample is T, or a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene only in the case where the base at position 3932 of the apolipoprotein E gene in a nucleic acid sample is C.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (18) to (22). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (18) to (22) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (18) to (21) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratio in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (18) to (20) (sets of nucleic acid for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (1) to (6):

(1) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is T and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose gene at position 3932 is C and of an antisense primer that specifically hybridizes a partial

region of the apolipoprotein E gene;

(2) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose base at position 1648 is A and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene whose gene at position 1648 is G and of an antisense primer that specifically hybridizes a partial region of the glycoprotein Ia gene;

(3) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene and which consists of an antisense primer that specifically hybridizes the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose base at position -863 is C and/or an antisense primer that specifically hybridizes the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose gene at position -863 is A and of a sense primer that specifically hybridizes a partial region of the tumor necrosis factor-α gene;

(4) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene whose base at position 825 is C and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene whose gene at position 825 is T and of an antisense primer that specifically hybridizes a partial region of the G-protein β3 subunit gene;

(5) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene whose base at position -482 is C and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene whose gene at position -482 is T and of an antisense primer that specifically hybridizes a partial region of the apolipoprotein C-III gene; and

(6) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -6 of the angiotensinogen gene and

which consists of an antisense primer that specifically hybridizes the partial DNA region containing the base at position -6 of the angiotensinogen gene whose base at position -6 is G and/or an antisense primer that specifically hybridizes the partial DNA region containing the base at position -6 of the angiotensinogen gene whose gene at position -6 is A and of a sense primer that specifically hybridizes a partial region of the angiotensinogen gene.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (1) to (6). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (1) to (6) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (1) to (5) (sets of nucleic acids for analyzing polymorphisms with five highest odds ratio and *P* values which are considered in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (1), (3), (4) and (5) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (7) to (11):

(7) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene whose base at position 1186 is G and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene whose gene at position 1186 is C and of an antisense primer that specifically hybridizes a partial region of the thrombospondin 4 gene;

(8) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene and which consists of an antisense primer that specifically hybridizes the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose base at position -863 is C and/or an antisense primer that specifically hybridizes the partial DNA region containing the base at position -863 of the tumor necrosis factor-α gene whose gene at position -863 is A and of a sense primer that

specifically hybridizes a partial region of the tumor necrosis factor-α gene;

(9) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 2136 of the thrombomodulin gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 2136 of the thrombomodulin gene whose base at position 2136 is C and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 2136 of the thrombomodulin gene whose gene at position 2136 is T and of an antisense primer that specifically hybridizes a partial region of the thrombomodulin gene;

(10) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 5713 of the thrombopoietin gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 5713 of the thrombopoietin gene whose base at position 5713 is A and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 5713 of the thrombopoietin gene whose gene at position 5713 is G and of an antisense primer that specifically hybridizes a partial region of the thrombopoietin gene; and

(11) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 994 of the platelet-activating factor acetylhydrolase gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 994 of the platelet-activating factor acetylhydrolase gene whose base at position 994 is G and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 994 of the platelet-activating factor acetylhydrolase gene whose gene at position 994 is T and of an antisense primer that specifically hybridizes a partial region of the platelet-activating factor acetylhydrolase gene.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (7) to (11). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (7) to (11) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (7) to (10) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratio in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (7) to (9) (sets of nucleic acids for analyzing polymorphisms

with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype comprising two or more sets of nucleic acids selected from the group consisting of the following (12) to (17);

(12) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 561 of the E-selectin gene and which consists of an antisense primer that specifically hybridizes the partial DNA region containing the base at position 561 of the E-selectin gene whose base at position 561 is A and/or an antisense primer that specifically hybridizes the partial DNA region containing the base at position 561 of the E-selectin gene whose gene at position 561 is C and of a sense primer that specifically hybridizes a partial region of the E-selectin gene;

(13) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene whose base at position 2445 is G and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene whose gene at position 2445 is A and of an antisense primer that specifically hybridizes a partial region of the fatty acid-binding protein 2 gene;

(14) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is C, and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is T, and of an antisense primer that specifically hybridizes a partial region of the glycoprotein Ibα gene;

(15) a set of nucleic acids consisting of a pair of primers which are designed to specifically amplify the partial DNA region containing a part of polymorphism at position -668 of the plasminogen activator inhibitor 1 gene, as well as a probe that specifically hybridizes the partial DNA region containing the sequence in the plasminogen activator inhibitor 1 gene in which four G successively exist in the 3' direction from the position -668 and/or a probe that specifically hybridizes the

partial DNA region containing the sequence in the plasminogen activator inhibitor 1 gene in which five G successively exist in the 3' direction from the position -668;

(16) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing a base at position 584 of the paraoxonase gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is G, and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is A, and of an antisense primer that specifically hybridizes a partial region of the paraoxonase gene; and

(17) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing a base at position 3932 of the apolipoprotein E gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is T, and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose gene at position 3932 is C, and an antisense primer that specifically hybridizes a partial region of the apolipoprotein E gene.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (12) to (17). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (12) to (17) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (12) to (16) (sets of nucleic acids for analyzing polymorphisms with five highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more nucleic acids from the group consisting of (12) to (15) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype, comprising two or more sets of nucleic acids selected from the group consisting of the following (18) to (22):

(18) a set of nucleic acids consisting of a pair of primers which are designed to specifically amplify the partial DNA region containing a part of polymorphism at position -668 of the plasminogen activator inhibitor 1 gene, as well as of a probe that specifically hybridizes the partial DNA region containing the sequence in the

plasminogen activator inhibitor 1 gene in which four G successively exist in the 3' direction from the position -668 and/or of a probe that specifically hybridizes the partial DNA region containing the sequence in the plasminogen activator inhibitor 1 gene in which five G successively exist in the 3' direction from the position -668;

(19) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene whose base at position -482 is C, and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene whose base at position -482 is T, and of an antisense primer that specifically hybridizes a partial region of the apolipoprotein C-III gene;

(20) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing a base at position 584 of the paraoxonase gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is G, and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 584 of the paraoxonase gene whose base at position 584 is A, and of an antisense primer that specifically hybridizes a partial region of the paraoxonase gene;

(21) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing a base at position 1018 of the glycoprotein Ibα gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is C, and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene whose base at position 1018 is T, and of an antisense primer that specifically hybridizes a partial region of region of the glycoprotein Ibα gene; and

(22) a set of nucleic acids which is designed to specifically amplify the partial DNA region containing a base at position 3932 of the apolipoprotein E gene and which consists of a sense primer that specifically hybridizes the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is T, and/or a sense primer that specifically hybridizes the partial DNA region containing the base at position 3932 of the apolipoprotein E gene whose base at position 3932 is C, and of an antisense primer that specifically hybridizes a partial

region of the apolipoprotein E gene.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (18) to (22). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (18) to (22) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (18) to (21) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (18) to (20) (sets of nucleic acid for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype comprising two or more sets of nucleic acids selected from the group consisting of the following (1) to (6);

(1) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at position 3932 is T and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at position 3932 is C and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the apolipoprotein E gene and that can specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene in concurrent use with the above first or second nucleic acid;

(2) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 1648 in the antisense strand of the glycoprotein Ia gene whose base at position 1648 is A and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 1648 in the antisense strand of the glycoprotein Ia gene whose base at position 1648 is G and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the glycoprotein Ia gene and that can specifically amplify the partial DNA region containing the base at position 1648 of the glycoprotein Ia gene in concurrent use with the above first or

second nucleic acid;

(3) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position -863 in the sense strand of the tumor necrosis factor α gene whose base at position -863 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position -863 in the sense strand of the tumor necrosis factor α gene whose base at position -863 is A and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the antisense strand of the tumor necrosis factor α gene and that can specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor α in concurrent use with the above first or second nucleic acid;

(4) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 825 in the antisense strand of the G-protein β3 subunit gene whose base at position 825 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 825 in the antisense strand of the G-protein β3 subunit gene whose base at position 825 is T and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the G-protein β3 subunit gene and that can specifically amplify the partial DNA region containing the base at position 825 of the G-protein β3 subunit gene in concurrent use with the above first or second nucleic acid;

(5) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position -482 in the antisense strand of the apolipoprotein C-III gene whose base at position -482 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position -482 in the antisense strand of the apolipoprotein C-III gene whose base at position -482 is T and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the apolipoprotein C-III gene and that can specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene in concurrent use with the above first or second nucleic acid; and

(6) a set of nucleic acids which consists of a first nucleic acid that specifically

hybridizes a partial region containing the base at position -6 in the sense strand of the angiotensinogen gene whose base at position -6 is G and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position -6 in the sense strand of the angiotensinogen gene whose base at position -6 is A and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the antisense strand of the angiotensinogen gene and that can specifically amplify the partial DNA region containing the base at position -6 of the angiotensinogen in concurrent use with the above first or second nucleic acid.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (1) to (6). However, kits may be constructed by making a group consisting of two or more sets of nucleic acids arbitrarily selected from (1) to (6) and selecting two or more sets of nucleic acids from such a group. For example, kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (1) to (5) (sets of nucleic acids for analyzing polymorphisms with five highest odds ratios and *P* values which are considered in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (1), (3), (4) and (5) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype comprising two or more sets of nucleic acids selected from the group consisting of the following (7) to (11);

(7) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 1186 in the antisense strand of the thrombospondin 4 gene whose base at position 1186 is G and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 1186 in the antisense strand of the thrombospondin 4 gene whose base at position 1186 is C and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the thrombospondin 4 gene and that can specifically amplify the partial DNA region containing the base at position 1186 of the thrombospondin 4 gene in concurrent use with the above first or second nucleic acid;

(8) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position -863 in the sense strand of

the tumor necrosis factor α gene whose base at position -863 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position -863 in the sense strand of the tumor necrosis factor α gene whose base at position -863 is A and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the antisense strand of the tumor necrosis factor α gene and that can specifically amplify the partial DNA region containing the base at position -863 of the tumor necrosis factor α in concurrent use with the above first or second nucleic acid;

(9) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 2136 in the antisense strand of the thrombomodulin gene whose base at position 2136 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 2136 in the antisense strand of the thrombomodulin gene whose base at position 2136 is T and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the thrombomodulin gene and that can specifically amplify the partial DNA region containing the base at position 2136 of the thrombomodulin gene in concurrent use with the above first or second nucleic acid;

(10) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 5713 in the antisense strand of the thrombopoietin gene whose base at position 5713 is A and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 5713 in the antisense strand of the thrombopoietin gene whose base at position 5713 is G and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the thrombopoietin gene and that can specifically amplify the partial DNA region containing the base at position 5713 of the thrombopoietin gene in concurrent use with the above first or second nucleic acid; and

(11) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 994 in the antisense strand of the platelet-activating factor acetylhydrolase gene whose base at position 994 is G and that is labeled with a first labeling substance, of a second nucleic acid that

specifically hybridizes a partial region containing the base at position 994 in the antisense strand of the platelet-activating factor acetylhydrolase gene whose base at position 994 is T and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the platelet-activating factor acetylhydrolase gene and that can specifically amplify the partial DNA region containing the base at position 994 of the platelet-activating factor acetylhydrolase gene in concurrent use with the above first or second nucleic acid.

In the above mention, kits are constructed by selecting two or more sets of nucleic acids from the group consisting of (7) to (11). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (7) to (11) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (7) to (10) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more sets of nucleic acids from the group consisting of (7) to (9) (sets of nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

A kit for detecting the genotype comprising two or more sets of nucleic acids selected from the group consisting of the following (12) to (17);

(12) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 561 in the sense strand of the E-selectin gene whose base at position 561 is A and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 561 in the sense strand of the E-selectin gene whose base at position 561 is C and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the antisense strand of the E-selectin gene and that can specifically amplify the partial DNA region containing the base at position 561 of the E-selectin gene in concurrent use with the above first or second nucleic acid;

(13) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base at position 2445 in the antisense strand of the fatty acid-binding protein 2 gene whose base at position 2445 is G and

that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base at position 2445 in the antisense strand of the fatty acid-binding protein 2 gene whose base at position 2445 is A and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the fatty acid-binding protein 2 gene and that can specifically amplify the partial DNA region containing the base at position 2445 of the fatty acid-binding protein 2 gene in concurrent use with the above first or second nucleic acid;

(14) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 1018 in the antisense strand of the glycoprotein Ibα gene whose base at position 1018 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 1018 in the antisense strand of the glycoprotein Ibα gene whose base at position 1018 is T and that is labeled with a second labeling substance, and of a third nucleic acid that specifically hybridizes a partial region in the sense strand of the glycoprotein Ibα gene and that can specifically amplify the partial DNA region containing the base at position 1018 of the glycoprotein Ibα gene in concurrent use with the above first or second nucleic acid;

(15) a set of nucleic acids which consists of a pair of nucleic acids (first and second nucleic acids) that is designed to specifically amplify the partial region of DNA containing a part of polymorphism at position -668 of the plasminogen activator inhibitor 1 gene, of a third nucleic acid that specifically hybridizes the nucleic acid which is obtained by amplification using plasminogen activator inhibitor 1 gene in which four G successively exist in the 3' direction from the position -668 as a template and the pair of nucleic acids, and of a fourth nucleic acid that specifically hybridizes a nucleic acid which is obtained by amplification using plasminogen activator inhibitor 1 gene in which five G successively exist in the 3' direction from the position -668 as a template and the pair of nucleic acids;

(16) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 584 in the antisense strand of the paraoxonase gene whose base at position 584 is G and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 584 in the antisense strand of the paraoxonase gene whose base at

position 584 is A and that is labeled with a second labeling substance, and of a third nucleic acid that specifically hybridizes a partial region in the sense strand of the paraoxonase gene and that can specifically amplify the partial DNA region containing the base at position 584 of the paraoxonase gene in concurrent use with the above first or second nucleic acid; and

(17) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at position 3932 is T and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at position 3932 is C and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the apolipoprotein E gene and that can specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene in concurrent use with the above first or second nucleic acid.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (12) to (17). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (12) to (17) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (12) to (16) (sets of nucleic acids for analyzing polymorphisms with five highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more nucleic acids from the group consisting of (12) to (15) (nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below).

A kit for detecting the genotype comprising two or more sets of nucleic acids selected from the group consisting of the following (18) to (22);

(18) a set of nucleic acids which consists of a pair of nucleic acids (first and second nucleic acids) that is designed to specifically amplify the partial region of DNA containing a part of polymorphism at position -668 of the plasminogen activator inhibitor 1 gene, of a third nucleic acid that specifically hybridizes the nucleic acid which is obtained by amplification using plasminogen activator inhibitor 1 gene in which four G successively exist in the 3’ direction from the

position -668 as a template and the pair of nucleic acids, and of a fourth nucleic acid that specifically hybridizes a nucleic acid which is obtained by amplification using plasminogen activator inhibitor 1 gene in which five G successively exist in the 3' direction from the position -668 as a template and the pair of nucleic acids;

(19) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position -482 in the antisense strand of the apolipoprotein C-III gene whose base at position -482 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position -482 in the antisense strand of the apolipoprotein C-III gene whose base at position -482 is T and that is labeled with a second labeling substance, and of a third nucleic acid that specifically hybridizes a partial region in the sense strand of the apolipoprotein C-III gene and that can specifically amplify the partial DNA region containing the base at position -482 of the apolipoprotein C-III gene in concurrent use with the above first or second nucleic acid;

(20) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 584 in the antisense strand of the paraoxonase gene whose base at position 584 is G and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 584 in the antisense strand of the paraoxonase gene whose base at position 584 is A and that is labeled with a second labeling substance, and of a third nucleic acid that specifically hybridizes a partial region in the sense strand of the paraoxonase gene and that can specifically amplify the partial DNA region containing the base at position 584 of the paraoxonase gene in concurrent use with the above first or second nucleic acid;

(21) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 1018 in the antisense strand of the glycoprotein Ibα gene whose base at position 1018 is C and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing a base corresponding to the base at position 1018 in the antisense strand of the glycoprotein Ibα gene whose base at position 1018 is T and that is labeled with a second labeling substance, and of a third nucleic acid that specifically hybridizes a partial region in the sense strand of the glycoprotein Ibα gene and that can specifically amplify the partial DNA

region containing the base at position 1018 of the glycoprotein Ibα gene in concurrent use with the above first or second nucleic acid; and

(22) a set of nucleic acids which consists of a first nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at position 3932 is T and that is labeled with a first labeling substance, of a second nucleic acid that specifically hybridizes a partial region containing the base corresponding to the base at position 3932 in the antisense strand of the apolipoprotein E gene whose base at position 3932 is C and that is labeled with a second labeling substance, and of the third nucleic acid that specifically hybridizes a partial region in the sense strand of the apolipoprotein E gene and that can specifically amplify the partial DNA region containing the base at position 3932 of the apolipoprotein E gene in concurrent use with the above first or second nucleic acid.

In the above mention, kits are constructed by selecting two or more nucleic acids from the group consisting of (18) to (22). However, kits may be constructed by making a group consisting of two or more nucleic acids arbitrarily selected from (18) to (22) and selecting two or more nucleic acids from such a group. For example, kits may be constructed by selecting two or more nucleic acids from the group consisting of (18) to (21) (sets of nucleic acids for analyzing polymorphisms with four highest odds ratios in Example mentioned below), or kits may be constructed by selecting two or more nucleic acids from the group consisting of (18) to (20) (nucleic acids for analyzing polymorphisms with three highest odds ratios in Example mentioned below).

In the above-mentioned kits, one or two or more of reagents (buffer, reagent for reaction, and reagent for detection, etc.) may be combined in response to the usage of the kit.

The present invention is hereinafter explained in more detail by way of Examples.

[Example 1] Selection of gene polymorphism

By using several kinds of public databases including PubMed [National Center for Biological Information (NCBI)], Online Mendelian inheritance in Men (NCBI), Single Nucleotide Polymorphism (NCBI), etc., from a comprehensive viewpoint including vascular biology, platelet-leukocyte biology, coagulation-

fibrinogenolysis system, and lipid and glucose metabolism, etc., 71 genes which were estimated to be associated with coronary arteriosclerosis, coronary artery spasm, hypertension, diabetes mellitus, hyperlipidemia, etc. were extracted from genes which had been previously reported. Furthermore, among the polymorphisms existing in these genes, 112 polymorphisms including polymorphisms which exist in promoter regions or exons, or polymorphisms which were located in splice donor sites or acceptor sites and expected to be associated with the functional changes of gene products were selected (Figures 1 and 2).

[Example 2] Determination of gene polymorphism

Subjects were 1869 Japanese men and women (1313 men and 556 women) who were hospitalized so as to undergo coronary angioplasty (balloon dilatation or stent implantation) between July 1998 and December 2001. The 1390 (910 in men and 480 in women) and 1001 (710 in men and 291 in women) coronary lesions undergone balloon dilatation and stent implantation, respectively, were examined. Follow-up coronary angiography was performed six months after the coronary angioplasty. Coronary restenosis lesions with acute occlusion after balloon dilatation or with subacute stent thrombosis were excluded from the study. Quantitative angiographic measurements were performed on end-diastolic frames. Restenosis was defined as narrowing of 50% or more of the minimal lumen diameter the coronary artery at the site coronary angioplasty was performed.

From each of the subjects, 7 mL of venous blood was collected in a tube containing 50 mmol/L EDTA-2Na and genome DNA was extracted by using a DNA extraction kit (Qiagen, Chatsworth, CA). Genotypes of single nucleotide polymorphisms were determined with a fluorescence- or colorimetry-based allele-specific primer-probe assay system (Toyobo Gene Analysis, Tsuruga, Japan) (see Figures 3 and 4). DNA fragment containing a polymorphism site was amplified by polymerase chain reaction (PCR) by using two kinds of allele specific sense primers (or antisense primers) whose 5' end were labeled with fluorescein isothiocyanate (FITC) or Texas red (TxR) and an antisense primer (or a sense primer) whose 5' end was labeled with biotin. Alternatively, DNA fragment containing polymorphism site was amplified by PCR by using two kinds of allele specific sense (or antisense) primers and an antisense (or a sense) primer whose 5' end was labeled with biotin, or by using a sense primer and an antisense primer

whose 5' end was labeled with biotin. The reaction solution (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 1 to 4 mmol/L of $MgCl_2$, 1 U of DNA polymerase (rTaq or KODplus; Toyobo Co., Ltd. Osaka, Japan) in corresponding DNA polymerase buffer. The amplification protocol comprised an initial denaturation at 95°C for 5 minutes; 35 to 45 cycles of denaturation at 95°C for 30 seconds, annealing at 55 to 67.5°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 2 minutes.

For determination of genotype by fluorescence, amplified DNA was incubated with a solution containing streptavidin-conjugated magnetic beads in 96-well plates at room temperature. The plates were placed on a magnetic stand, supernatants were collected from the wells and then transferred to the wells of a 96-well plate containing 0.01 M NaOH, followed by measuring fluorescence by microplate reader at excitation wavelength and emission wavelength of 485 nm and 538 nm for FITC and at excitation wavelength and emission wavelength of 584 nm and 612 nm for TxR. Furthermore, for determination of genotype by colorimetry, amplified DNA was denatured with 0.3 M NaOH and then subjected to hybridization at 37°C for 30 min in hybridization buffer containing 30 to 45% formamide with any of allele-specific capture probes fixed to the bottom of the wells of a 96-well plate. After thorough washing of the wells, alkaline phosphatase–conjugated streptavidin was added to each well and the plate was shaken at 37°C for 15 min. The wells were again washed, and, after the addition of a solution containing 0.8 mM 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (monosodium salt) and 0.4 mM 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt, the absorbance at 450 nm was measured.

To confirm the accuracy of genotyping by this method, DNA samples of 50 people were selected at random, and the samples were subjected to PCR-restriction fragment length analyzing polymorphism method or direct sequencing method of PCR products. In any samples, the genotype determined by the allele specific primer-probe measurement system was identical to that determined by PCR-polymerase chain reaction-restriction fragment length polymorphism method or direct sequencing method.

Note here that statistical analysis in the following association study was

carried out as follows. Clinical data were compared between coronary lesions with restenosis and without restenosis by the unpaired Student's *t* test or the Mann-Whitney *U* test. Qualitative data were examined by the chi-square test. Allele frequencies were estimated by the gene counting method, and the chi-square test was used to identify departures from Hardy-Weinberg equilibrium. The present inventors performed multivariate logistic regression analysis with adjustment for risk factors, with restenosis as a dependent variable and with age, body mass index (BMI), smoking status (0 = nonsmoker, 1 = smoker), metabolic variables (0 = no history of diabetes mellitus, hypercholesterolemia, or hyperuricemia; 1 = positive history) and genotype of each polymorphism as independent variables. Each genotype was assessed according to dominant, recessive, and additive genetic models, and the *P* value, odds ratio, and 95% confidence interval were calculated. For combined genotype analyses, the present inventors performed the stepwise forward selection method of multivariate logistic regression analysis to calculate odds ratios for each combined genotype.

[Example 3] Selection of polymorphism associated with restenosis after coronary angioplasty and development of method for diagnosing restenosis after coronary angioplasty

The present inventors performed an association study of the 112 polymorphisms of the 71 candidate genes with myocardial infarction in 451 men (myocardial infarction: 219, control: 232) and in 458 women (myocardial infarction: 226, control: 232) in the previous report (Yamada Y, Izawa H, Ichihara S, et al. Genetic risk diagnosis system for myocardial infarction developed by a large scale association study of 112 gene polymorphisms in 5061 individuals (in press)). In this study, the present inventors have found that 19 and 18 single nucleotide polymorphisms were associated with the development of myocardial infarction in men and women, respectively, which included candidate genes of restenosis after coronary angioplasty (see Figures 1, 2 and 5). In this Example, a large scale association study on the association of these single nucleotide polymorphisms with restenosis after balloon dilatation or in-stent restenosis was carried out in total 2391 coronary lesions.

Figures 6 and 7 show background data of all of the examined 2391 coronary lesions (1620 for men, 771 for women). For men, the prevalence of hypertension

and diabetes mellitus was significantly greater, and age was significantly smaller, in coronary lesions with restenosis after balloon angioplasty than in those without restenosis; and the prevalence of smoking, diabetes mellitus and hyperuricamia was significantly greater, and age was significantly smaller, in coronary lesions with restenosis after stent implantation than in those without restenosis (Figure 6). For women, age and the prevalence of smoking and diabetes mellitus were significantly greater in coronary lesions with restenosis after balloon angioplasty than in those without restenosis; and age and the prevalence of diabetes mellitus were significantly greater, and the prevalence of smoking, hypertension and hyperuricemia was significantly smaller in coronary lesions with restenosis after stent implantation than in those without restenosis (Figure 7). Also, for women, a prevalence of right coronary artery was significantly higher, and that of left circumflex artery was significantly lower, among coronary lesions with restenosis after balloon angioplasty than among those without restenosis; and the prevalence of left anterior descendent artery was significantly higher among coronary lesions with restenosis after stent implantation than among those without restenosis (Figure 7).

In the association study of restenosis after coronary angioplasty with 19 polymorphisms for men and 18 polymorphisms for women, multivariate logistic regression analysis with adjustment for age, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, hypercholesterolemia and hyperuricemia revealed that six and five polymorphisms were associated significantly with restenosis after balloon dilatation and stent implantation, respectively ($P < 0.05$ in either a dominant or recessive genetic model) (Figure 8 shows the data for men and Figure 9 shows the data for women).

The present inventors performed the stepwise forward selection method of multivariate logistic regression analysis (Figures 10 and 11). In this method, either a dominant or recessive model was used based on the P values (lower P values) for association of each polymorphism with restenosis after coronary angioplasty shown in Figures 8 and 9. The chromosomal loci of these genes are also shown in Figures 10 and 11. Although the loci of the tumor necrosis factor-α gene and the platelet-activating factor acetylhydrolase gene are located near each other, no association in distribution between the both polymorphisms was observed. Similarly, in spite of the proximity of the plasminogen activator inhibitor-1 gene and the paraoxonase gene, no association in distribution between the both

polymorphisms was observed. Odds ratios restenosis after balloon dilatation or stent implantation based on combined genotypes with the stepwise forward selection method is shown in Figures 12, 13 and 16A for men and in Figures 14, 15 and 16B for women. For men, combined genotype analysis of five polymorphisms (ApoE (3932T→C) polymorphism, GPIa (1648A→G) polymorphism, TNFα (-863C→A) polymorphism, G-protein β3 (825C→T) polymorphism and ApoC-III (-482C→T) polymorphism) revealed that the maximal odds ratio for restenosis after balloon dilatation was 10.55 (Figures 12 and 16A). Further combination with the remaining polymorphism (AGT (-6G→A) polymorphism) for a total of six polymorphisms, revealed that the maximal odds ratio for restenosis after balloon dilatation was 15.09 (Figure 16A). Also, among men, combined genotype analysis of five polymorphisms (TSP4 (1186G→C) polymorphism, TNFα (-863C→A) polymorphism, TM (2136C→T) polymorphism, TPO (5713A→G) polymorphism and PAF-AH (994G→T) polymorphism) revealed that the maximal odds ratio for in-stent restenosis was 6.64 (Figures 13 and 16A). For women, combined genotype analysis of five polymorphisms (E selectin (561A→C) polymorphism, FABP2 (2445G→A) polymorphism, GPIbα (1018C→T) polymorphism, PAI1 (-668/4G→5G) polymorphism and PON (584G→A) polymorphism) revealed that the maximal odds ratio for restenosis after balloon dilatation was 37.43 (Figures 14 and 16B). Further combination with the remaining polymorphism (ApoE (3932T→C) polymorphism) for a total of six polymorphisms, revealed that the maximal odds ratio for restenosis after balloon dilatation was 44.54 (Figure 16B). Also, among women, combined genotype analysis of five polymorphisms (PAI1 (-668/4G→5G) polymorphism, ApoC-III (-482C→T) polymorphism, PON (584G→A) polymorphism, GPIbα (1018C→T) polymorphism and ApoE (3932T→C) polymorphism), revealed that the maximal odds ratio for in-stent restenosis was 117.83 (Figure 15 and 16B).

As mentioned above, according to the multivariate logistic regression analysis, for men and women, six single nucleotide polymorphisms are associated with restenosis after balloon dilatation and five single nucleotide polymorphisms are associated with in-stent restenosis. That is to say, the present inventors have examined the association of restenosis after coronary angioplasty with 19 single nucleotide polymorphisms for men and 18 single nucleotide polymorphisms for women, and shown that six and five single nucleotide polymorphisms were

associated with restenosis after balloon dilatation and in-stent stent restenosis, respectively, in men and women, by a large-scale association study with 2391 coronary lesions. Furthermore, the present inventors developed a genetic risk diagnosis system for restenosis that yielded maximal odds ratios of 15.09 and 44.54 for restenosis after balloon dilatation, and of 6.64 and 117.83 for in-stent restenosis in men and women, respectively, on the basis of the stepwise forward selection method of multivariate logistic regression analysis.

The major cause of restenosis after balloon dilatation is chronic remodeling of coronary arteries and that of in-stent restenosis is neointimal hyperplasia (Mintz GS, Popma JJ, Pichard AD, et al. Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study. Circulation 1996;94:35-43.; Hoffmann R, Mintz GS, Dussaillant GR, et al. Patterns and mechanisms of in-stent restenosis. A serial intravascular ultrasound study. Circulation 1996;94:1247-54). The present inventors thus examined the association between 19 single nucleotide polymorphisms for men and 18 single nucleotide polymorphisms for women and restenosis after coronary angioplasty on the basis of a comprehensive overview of vascular biology, platelet and leukocyte biology, fibrinolysis system, as well as lipid and glucose metabolism and other metabolic factors. Indeed, genes associated with restenosis played roles in diverse aspects of the etiology of this condition. The genes associated with restenosis after balloon dilatation played roles in vascular biology (G-protein β3 subunit and E-selectin) and inflammation (tumor necrosis factor-α), hypertension (angiotensinogen), lipid metabolism (apolipoproteins E and C-III, fatty acid-binding protein 2 and paraoxonase), platelet function (glycoprotein Ia and glycoprotein Ibα), and fibrinolysis (plasminogen activator inhibitor-1), etc. Also, genes associated with in-stent restenosis played roles in vascular biology (thrombospondin 4) and inflammation (tumor necrosis factor-α and platelet-activating factor acetylhydrolase), lipid metabolism (apolipoproteins E and C-III and paraoxonase), platelet function (thrombomodulin, thrombopoietin and glycoprotein Ibα), and fibrinolysis (plasminogen activator inhibitor-1), etc. One polymorphism (tumor necrosis factor-α gene) was associated with both restenosis after balloon dilatation and in-stent restenosis in men, and four polymorphisms (plasminogen activator inhibitor-1 gene, paraoxonase, glycoprotein Ibα and apolipoproteins E genes) in women. The maximal odds ratios of restenosis after coronary angioplasty (in restenosis after balloon dilatation, 15.09 for men and 44.54

for women; and in in-stent restenosis, 6.64 for men and 117.83 for women) obtained with our genetic risk diagnosis system were the highest among such values previously reported by association studies of restenosis.

Among 15 polymorphisms associated with restenosis after coronary angioplasty, the polymorphisms of apolipoprotein E (van Bockxmeer FM, Mamotte CDS, Gibbons FR, Taylor RR. Apolipoprotein e4 homozygosity-a determinant of restenosis after coronary angioplasty. Atherosclerosis 1994; 110:195-202.), angiotensinogen (Volzke H, Hertwig S, Rettig R, Motz W. The angiotensinogen gene 235T variant is associated with an increased risk of restenosis after percutaneous transluminal coronary angioplasty. Clin Sci 2000;99:19-25), plasminogen activator inhibitor-1 (Ortlepp JR, Hoffmann R, Killian A, Lauscher J, Merkelbach-Brese S, Hanrath P. The 4G/5G promoter polymorphism of the plasminogen activator inhibitor-1 gene and late luminal loss after coronary stent placement in smoking and nonsmoking patients. Clin Cardiol 2001; 24: 585-591), and E-selectin (Rauchhaus M, Gross M, Schulz S, et al. The E-selectin SER123ARG gene polymorphism and restenosis after successful coronary angioplasty. Int J Cardiol 2002;83:249-257) were previously shown to be associated with restenosis. In contrast to our results, restenosis was not associated with the polymorphisms of the glycoprotein Ia gene (von Beckerath N, Koch W, Mehilli J, et al. Glycoprotein Ia C807T polymorphism and risk of restenosis following coronary stenting. Atherosclerosis 2001;156:463-468) or G-protein β3 subunit gene (von Beckerath N, Kastrati A, Koch W, et al. G protein β3 subunit polymorphism and risk of thrombosis and restenosis following coronary stent placement. Atherosclerosis 2000; 149: 151-155), although these genes may be important in the etiology of restenosis (Matsuno H, Kozawa O, Niwa M, Uematsu T. Inhibition of von Willebrand factor binding to platelet GP Ib by a fractionated aurintricarboxylic acid prevents restenosis after vascular injury in hamster carotid artery. Circulation 1997;96:1299-304.; Iaccarino G, Smithwick LA, Lefkowitz RJ, Koch WJ. Targeting Gβγsignaling in arterial vascular smooth muscle proliferation: a novel atrategy to limit restenosis. Proc Natl Acad Sci USA 1999;96:3945-50.). Other nine polymorphisms have not been examined on association with restenosis after coronary angioplasty. Among them, tumor necrosis factor-α (Clausell N, de Lima VC, Molossi S, et al. Expression of tumor necrosis factor α and accumulation of fibronectin in coronary artery restenotic lesions retrieved by atherectomy. Br Heart J 1995, 73:534-9) and glycoprotein Ibα

(Gawaz M, Neumann FJ, Ott I, May A, Rudiger S, Schomig A. Changes in membrane glycoproteins of circulating platelets after coronary stent implantation. Heart 1996; 76: 166-72.) are thought to have roles in the development of this condition.

It is possible that some of the polymorphisms examined in the Example are in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of restenosis after coronary angioplasty. The results by the present inventors indicate, however, that ten and seven genes are susceptibility loci for restenosis after coronary angioplasty in Japanese men and women, respectively, and that the corresponding combined genotypes may be useful for the diagnosis of genetic risk for restenosis after balloon dilatation or in-stent restenosis. It is thought that the genetic risk diagnosis system of the present invention will contribute to the prediction of restenosis after coronary angioplasty and provide information on the selection of the most appropriate treatment, and thereby quality of life for individuals with coronary artery disease is improved as well as medical expenses is reduced.

The present invention is not limited to the description of the above embodiments. A variety of modifications, which are within the scopes of the following claims and which are achieved easily by a person skilled in the art, are included in the present invention.

INDUSTRIAL APPLICABILITY

According to the present invention, gene polymorphism associated with restenosis after coronary angioplasty is analyzed and the genotype of nucleic acid sample is detected. By using the information about polymorphism obtained by the detection of the genotype, diagnosis of the risk of restenosis after coronary angioplasty with high accuracy and high predictability can be carried out. That is to say, the present invention provides an effective means for understanding in advance the risk of development of restenosis after a certain coronary angioplasty. Therefore, the present invention provides information useful for selecting an appropriate treatment method and enables an appropriate treatment method to be selected. Thus, high effect of treatment can be realized and the quality of life of patients with coronary artery disease can be improved. In addition, it is possible to

solve the problem such as an increase in medical expenses because of the repetition of inappropriate treatments, thus contributing much to the medical economy. On the other hand, the present invention provides information useful in clarifying the development mechanism of restenosis, so that it provides an extremely important means for establishing prevention method of restenosis.